EXTERNAL SCIENTIFIC REPORT

Inventory of available data and data sources and proposal for data collection on vector-borne zoonoses in animals[1]

Prepared by

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ABSTRACT

An inventory of data collected from animals was carried out on the following agents of vector borne zoonoses: *Borrelia burgdorferi* sensu lato (sl), West Nile virus (WNV), *Francisella tularensis*, *Leptospira*, Hantavirus, *Leishmania infantum*, tick borne encephalitis virus (TBEV) and Crimean Congo hemorrhagic fever virus (CCHFV). The role of animals in transmission cycles of these agents is described through a narrative literature review. Information on data collection carried out by competent authorities of European Union Member States, Norway, and Switzerland was gathered using questionnaires. Ecological and epidemiological research was analysed through a systematic literature review. Fourteen out of 29 countries replied to the questionnaires. Results show that, up to 2009, data collection by competent authorities was relatively frequent on *Leptospira*, *F. tularensis*, and WNV. Conversely, data collection on *B. burgdorferi* sl in animals was less frequent, in spite of a widespread and expanding distribution of this agent. Based on information gathered in the data inventory, proposals were developed for data collection within the framework of Directive 2003/99/EC. The following animal species were proposed in data collection on one or more agent: dogs (which were proposed in data collection on *B. burgdorferi* sl, *L. infantum*, *Leptospira*, TBEV, *F. tularensis*, and Hantavirus), domestic ruminants (*B. burgdorferi* sl, TBEV, *F. tularensis*, CCHFV, and *Leptospira*), horses (*B. burgdorferi* sl, *F. tularensis*, WNV, and *Leptospira*), pigs (*F. tularensis* and *Leptospira*), wild boars and foxes (*F. tularensis* and *Leptospira*), wild and synanthropic rodents (*Leptospira*, Hantavirus, TBEV, and *F. tularensis*), hares (*F. tularensis* and CCHFV), wild and synanthropic birds (WNV), and farmed ostriches (CCHFV).

KEY WORDS

Borrelia burgdorferi, West Nile virus, Francisella tularensis, Leptospira, Hantavirus, Leishmania infantum, Tick-borne Encephalitis virus, Crimean Congo Hemorrhagic Fever virus, data collection, vector borne zoonosis.

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SUMMARY

In this report, science-based recommendations were generated for the inclusion of agents of vector borne zoonoses in animals in the reporting system on zoonoses at the EU level, within the framework of Directive 2003/99/EC[2]. The considered agents include *Borrelia* spp., West Nile virus, *Francisella* spp., *Leptospira* spp., Hantavirus, Tick Borne Encephalitis virus, *Leishmania* spp. and Crimean-Congo Haemorrhagic Fever virus.

The basis for generating recommendations and detailed technical specifications was obtained through an inventory of available data, including:

a narrative literature review of each agent's transmission cycle (focussing on the role of animals), the diagnosis of infection in animals, clinical signs, and antibody response;

the collection of information on current and past data collection, concerning each agent in animals, by competent national authorities in each EU member state, and Norway and Switzerland, through questionnaires submitted to members of the EFSA Task Force on Zoonoses data collection;

a systematic literature review of publications including research on data collection from other institutions, by searching online data bases.

Based on the results of the data inventory, a stepwise process was adopted to produce proposals for data collection in animals. First, a comprehensive list of animal species involved in each agent's transmission cycle was built. Species to be included in data collection were subsequently selected by applying specific criteria.

Specifications on data collection on selected species were given as follows: subpopulations to be covered; agents and possible subtypes; sampling context and design, sampling stage; type of specimen to be taken and sampling technique; diagnostic methods to be used; recommendations for data submission.

Dynamic, mathematical models were built for a subset of agents from available information on certain parameters, and including agents transmitted by ticks (*Borrelia burgdorferi* sensu lato), by insects (*Leishmania infantum*; a descriptive model was built for West Nile virus), or also by direct routes or the environment (*Francisella tularensis*). These models were used to point out essential transmission features and to explore the relationship between data collected on animals (i.e. prevalence of agents, or incidence of clinical cases) and the intensity of transmission of agents in the source of infection for animals and man (i.e. vectors or reservoir hosts). Key parameters and gaps in knowledge were identified, including rates of contact between sources of infections and animals, antibody response and decay, incidence of clinical disease. The model for *B. burgdorferi* sl was used as a general guide for the determination of the sample size needed to detect, as statistically significant, epidemiologically meaningful trends in the prevalence of antibodies in animals.

Completed questionnaires on current and past data collection activities, on the selected zoonotic agents in animals, were received from 14 out of the 29 considered countries. These included Denmark, Estonia, Finland, France, Hungary, Italy, Latvia, Norway, Portugal, Romania, Slovakia, Slovenia, Sweden, and Switzerland. Data collection by competent authorities of those countries was more frequent on those agents that have been long known as causes of disease in both humans and animals, such as, for example, *Leptospira* spp. and *F. tularensis*. Moreover, data collection is carried out in several countries on West Nile virus (WNV), which is a recently emerging agent that causes disease in humans and horses in Europe. On the other hand, data collection on *B. burgdorferi* sl in animals is less

[2] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC (OJ L 325, 12.12.2003 p. 31)

frequent, in spite of a relatively high incidence of infection in humans and animals in certain countries, and of a widespread and expanding distribution of the agent and of the tick vectors.

Results of the narrative literature review confirmed the central role of animals, acting as reservoirs and amplifying hosts, in the ecological mechanisms underlying maintenance and transmission of agents of vector borne zoonoses. On the other hand, animals can be dead-end hosts when their role in transmission is negligible. Based on the possibility of detection of infection, mostly based on accurate methods for laboratory diagnosis, both reservoir and dead end hosts can be used in data collection.

In the systematic literature review, publications from data collection on the selected agents in animals were retrieved (data collection on agents in vectors was not considered). A ten year range (2000 – 2010) was initially used, but the literature search was extended to 20 years if no publication was initially found for certain countries. By means of data extracted from publications, prevalence and 95% confidence interval of each agent were calculated by animal species, country, and for groups of countries, taking into account potential non independence of the results obtained within the same data collection activity (often carried out in single geographic locations). Such prevalence estimates were cautiously interpreted. In fact, retrieved publications mostly reported research results and, consequently, data collection was affected by variable research objectives, and by heterogeneous sampling designs and diagnostic methods. As a consequence, prevalence estimates could not be considered as representative considering the level of animal species or countries. On the other hand, the critical evaluation of results from the systematic literature review was useful for generating proposals for data collection. In fact, retrieved publications included detailed description of methods used in sampling and laboratory analysis. Morevoer, prevalence of agents in animals provided information on real scenarios in Europe that was useful in the selection of animal species for data collection.

A major distinction was made between active and passive data collection. In the definition that we adopted in this report, active data collection consists of sampling animals from the target population according to a pre-established design, regardless of the occurrence of clinical signs, whereas passive data collection consists of reporting suspect cases (animals showing defined clinical signs) and the subsequent execution of confirmatory laboratory diagnosis .

Proposals for data collection preferably included animal species sharing habitat frequentation with people and that can, therefore, be used as indicators of human exposure. Accordingly, active and passive data collection on dogs is proposed for *B. burgdorferi* sl, *L. infantum*, *Leptospira*, TBEV, whereas only active data collection on dogs is proposed for *F. tularensis* and Hantavirus.

Farm animals that are routinely objectives of data collection for these or other agents would be the most cost effective data sources. Domestic ruminants (cattle, but also sheep and goats) are proposed for active data collection on *B. burgdorferi* sl, TBEV, *F. tularensis*, CCHFV, and for passive data collection on *Leptospira*.

Active data collection on horses is proposed for *B. burgdorferi* sl, *F. tularensis*, WNV; passive data collection is proposed for *Leptospira* and WNV.

Domestic pigs can be used in active data collection on *F. tularensis* and in passive data collection on *Leptospira* (on farm, and at the slaughterhouse). Active data collection on *F. tularensis* and *Leptospira* spp. is also proposed for hunted wild boars and foxes.

Wild and synanthropic birds are proposed for active and passive data collection on WNV.

Hares (*Lepus europaeus*) are proposed in active data collection on *Francisella* (at the export and hunting stages) and CCHFV (at export), and in passive data collection on *Francisella*.

Farmed ostriches are proposed for active data collection on CCHFV.

Wild and synanthropic rodents are known reservoir hosts for several agents but access to these species is much more difficult compared to domestic animals. We nevertheless include proposals for capture for active data collection on *Leptospira*, Hantavirus, TBEV, and passive data collection on *F. tularensis*.

Stratification of sampling at the first or second order administrative divisions within each country is generally proposed at the sampling and at reporting stage.

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BACKGROUND

The European Union (EU) system for monitoring and collection of information on zoonoses is established by Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents (EC 2003). Eight zoonoses were originally considered in the data collection. Nevertheless, further zoonoses can be included based upon the epidemiological situation in the EU or in certain Member States (MSs), according to Annex I, section B, point 4 of Directive 2003/99/EC. The existence of a network of representatives of each MS collaborating with EFSA in data collection on zoonoses, and of a well established system for data submission and subsequent management and analysis further support the inclusion of new zoonotic agents among those that are the objects of data collection.

During the last few decades an increase in emerging and re-emerging vector-borne zoonoses has been observed in the EU and neighbouring countries (Blancou et al., 2005[3]; Report by the WHO/FAO/OIE 2004[4]; Vorou et al., 2007[5]). Data on vector-borne zoonoses cases in humans are provided through the Community networks for the epidemiological surveillance and control of communicable diseases established under Decision No 2119/98/EC run by ECDC. On the other hand, in spite of the critical role of animals in the transmission of these agents besides their well known role as sources of useful data on the agents' occurrence, data from animals are not officially collected and analysed at the European level.

TERMS OF REFERENCE

The purpose of the assignment is to:

investigate the availability of data on vector-borne zoonoses in animals in the EU, excluding the data on the arthropod vectors,

analyse the characteristics of this information,

elaborate a proposal for possible collection of these data by the EU Member States.

The following vector-borne agents are to be covered: *Francisella tularensis*, Hantaviruses, *Leptospira* spp., *Borrelia* spp., Tick-borne Encephalitis virus group, *Leishmania* spp., West Nile virus, Crimean Congo Haemorrhagic Fever virus.

Inventory of available data and data sources

The inventory of the available data on the occurrence of the above listed agents in animals, in the MSs should cover the following information:

identification of the MS in question, organisations involved in the collection and monitoring, sources of the data if available, animal species covered, and estimations of the quantity of the data and their characterization (e.g. number of years covered, number of data records);

information on the role of different animal species as reservoirs for the listed vector-borne agents when available;

information on occurrence and geographical distribution of the agents, their hosts and vectors, if information is available;

description and design of the current or past national or local systems for monitoring.

[3] Blancou J, Chomel BB, Belotto A, Meslin FX, 2005. Emerging or re-emerging bacterial zoonoses: factors of emergence, surveillance and control. Vet. Res. 36, 507-522.

[4] http://whqlibdoc.who.int/hq/2004/who_cds_cpe_zfk_2004.9.pdf

[5] Vorou RM, Papavassiliou VG, Tsiodras S, 2007. Emerging zoonoses and vector-borne infections affecting humans in Europe. Epidemiol Infect. 135, 1231-1247.

This inventory will be made through a detailed literature review including published scientific articles, national and international reports, other documents and data published on web sites.

Proposal for data collection on occurrence of the agents in animals

The proposal should identify the agents and the animal species where the data collection is feasible and useful for the risk managers and assessors at the EU level under the Directive 2003/99/EC. This proposal should also describe the type of information to be recorded and reported by Member States under the Directive 2003/99/EC, together with the rationale for the choices made in this proposal.

These reporting schemes should be feasible in all EU MSs and could, in particular, specify:

Animal species and sub-population to be covered;

Agent species and possible subtypes;

Sampling context and design (i.e. monitoring, survey, clinical investigation, etc.);

Sampling stage (farm, slaughterhouse, etc.);

Type of specimen taken and sampling technique;

Diagnostic/analytical method to be used.

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INTRODUCTION AND OBJECTIVES

INTRODUCTION

Vector borne zoonoses are emerging threats to public health in Europe. In fact, the geographic ranges of several agents of these diseases are expanding and their frequency is increasing over time. Spatial and temporal trends of the frequency of microbial agents in animals and humans, and the emergence of vector borne zoonoses are affected by multiple causes, including environmental factors (i.e. climate change, growing populations of animal reservoir hosts), changes in land use by man, greater movements of people and animals. Moreover, mutations of the agents can expand host range or augment pathogenicity.

In order to take decisions in the prevention and control of these diseases, it is indispensable to obtain information on spatial and temporal trends of the frequency of microbial agents. As a consequence, continuous data collection on agents of vector borne zoonoses should be a priority not only for individual countries (whose borders can be crossed by these agents in several ways), but also for the whole of Europe. In fact, harmonisation of data collection and analysis at the EU level is needed to draw useful conclusions, and guide effective prevention and control.

Animals serve as hosts for vector borne agents and for arthropod vectors even though their role in each agent's transmission varies greatly. In general, animals are more likely than humans to be exposed to agents of vector borne zoonoses, and the infections can be detected by clinical and pathological signs, and by available laboratory diagnosis. Under these circumstances, data collection from animals may provide a unique opportunity to evaluate occurrence and intensity of transmission of the agents of vector borne zoonoses. Moreover, through appropriate sampling design, statistical analysis, and critical interpretation of results, data from animals can be useful in following temporal and spatial trends of the frequency of these agents, and in the early detection of their introduction into previously free geographic areas.

OBJECTIVES

The main objectives of this report were to

gather available data on vector borne agents in animals, and

define proposals for data collection on these in animals, that will be considered by EFSA for inclusion in the annual data collection according to the Directive 2003/99/EC.

The specific objectives of the data inventory were to:

classify animal species based on their role in each agent's transmission cycle,

review options for the diagnosis of infection in animals,

gather information on past and present status of data collection on agents of vector borne zoonoses in animals by competent authorities in the EU, Norway and Switzerland, and

summarise results of data collected in animals for research purposes.

The specific objectives of the proposals for data collection were to:

present several options for data collection on each agent, and

guide the harmonised data collection within a country and across countries, considering differences in ecological and epidemiological situations.

DEFINITIONS

In the scope of this report the following definitions were considered:

Accidental host: this term has been used to classify animals in the transmission cycle of *Leptospira*, to indicate an animal species that is susceptible to the agent but has a negligible role in the subsequent transmission of the agent to other animals or the environment. This definition is, therefore, equivalent to dead-end host. In the case of *Leptospira*, accidental hosts may suffer severe clinical and pathological consequence of the infection.

Active data collection: the process of sampling animals (or other sampling units) from a study population, according to an established sampling design, regardless of the presence of clinical signs or pathological lesions. Sampled units are subsequently examined (most often by laboratory tests) and

classified as positive or negative for an agent of vector borne zoonoses. Even data collection activites that are carried out only once are included in this definition, as opposed to active surveillance where data collection is typically continued over time or periodic. Some examples of data collection encountered in this report which were classified as active data collection are briefly described here. The sampling of animals which were originally selected for different purposes, and the subsequent testing for a specific agent of interest, is considered here as active data collection. As an example, testing for the presence of antibodies against agents of vector borne zoonoses, of sera from cattle that were originally collected within a official testing program for brucellosis is considered as active data collection. It must be, however, noted that estimates of prevalence of vector borne agents obtained from this sample may be biased, if testing for brucellosis is not carried out based on a random sampling from the cattle population. Sampling wild animals which were killed during hunting, to be tested for agents of vector borne zoonoses is also included in active data collection. Even in this case, prevalence estimates may be biased since hunted animals (the study population) are unlikely to be a random sample of the target population. Sampling wild animals captured or killed in population control activities. Capturing wild animals for the specific purpose of testing them for agents of vector borne zoonoses, and also sampling wild animals captured for different studies, for example, by mammologists. Sampling healthy animals after an outbreak of an agent of vector borne zoonoses in humans or in animals is also considered as active data collection.

Administrative divisions: smaller units in which countries are divided, that are characterised by a certain degree of autonomy. Largest units within a country are definied first order administrative divisions that, in turn, can be divided into second order administrative divisions.

Amplifying host: an animal species that does not play the main role in the maintenance of a microbial agent in nature (reservoir or maintenance host), but may undergo active infection with intense multiplication of the agent. As a consequence, amplifying hosts may serve as sources of an agent for the environment, vectors, other animals, and humans.

Confidence interval: the range of values expressing the uncertainty in the estimate of a parameter (such as the prevalence of a vector borne agent) in a sample of units (i.e. animals) from a population. Based on a practical definition, the confidence interval would include the true prevalence in the population, with a certain confidence. The confidence interval is affected by the sample size: large samples provide narrow confidence intervals and, therefore, less uncertainty in the estimate. Furthermore, when data from several studies (i.e. data collection activites) are combined, greater variability of prevalence obtained in different studies corresponds to a wider confidence interval of the overall prevalence estimate (see section on systematic literature review).

It is to be noted that, in most publications reviewed in this report, data collection was carried out at specific location and for specific research purposes. As a consequence, even when relatively large samples of animals were tested, results are to be carefully interpreted, and conclusions cannot be drawn on the prevalence of vector borne agents in animal species, in entire countries or groups of countries.

Dead-end host: an animal species that is susceptible to infection by an agent of vector borne zoonosis but with a negligible role in the subsequent transmission of the agent to vectors, the environment, or other animals.

Direct laboratory diagnosis: includes those techniques aimed at the detection of the agent or its parts (including genetic material, antigens) in animal tissues.

Indirect laboratory diagnosis: includes those techniques aimed at the detection of the response of the animal to infection by an agent, most commonly, the detection of antibodies against the agent.

Notifiable agent: an agent for which any case of infection in animals is required by law to be reported to the health authorities. The fact that an agent is notifiable does not imply that a passive data

collection is carried out (see below). In fact, even cases of infection detected by laboratory diagnoses in clinically healthy individuals are to be notified.

Passive data collection: is the reporting of suspect cases of disease, based on clinical signs or death, to be submitted for subsequent confirmation of infection by laboratory diagnosis. Criteria for the definition of suspect cases and for confirmation are established. The competent organization "passively" receives and stores the data. In this report, the following data collection activities are classified as passive data collection: reporting of suspect cases of an agent of vector borne zoonoses by owners, private or public veterinarians; reporting dead wild animals, of a certain species, by personnel frequenting an outdoor environment for reasons unrelated to data collection on vector borne agents, such as hunters, foresters, environmental police. Based on a definition by Thrustfield (2006)[6], passive data collection includes the collection of results of tests for specific agents in animals, carried out in diagnostic laboratories. The heterogeneity of reasons for submission and the lack of information on sampling design make this type of data collection very prone to bias.

Parallel testing: the diagnostic process based on the execution of two (or more) diagnostic procedures for the same agent, on the same individual that is classified as positive if at least one of the procedures yields positive results. Parallel testing is carried out with the purpose of increasing diagnosis sensitivity, which is more likely if two independent tests are used, such as, direct and indirect tests for the same agent. Parallel testing might, however, reduce specificity.

Reservoir host: an animal species in which the agent normally lives and multiplies. A reservoir host acts as a source of infection for other animals, including humans and ensures long term maintenance of pathogens in an area. Traditionally, when referring to animals involved in the transmission cycle of *Leptospira*, reservoir hosts are defined as **maintenance hosts**. This term expresses the role of certain animal species in ensuring survival and perpetuation of the agent. According to some authors, the term reservoir hosts also includes a role in transmission to susceptible species (such as humans, for agents of zoonoses).

Sample: a set of units (animals, herds, land units) that are selected from a larger, defined population. Observations (i.e. diagnostic tests) are carried out on elements of the sample, in order to draw conclusions on the population at large (target population).

Sampling: the process of selection of the sample units. **Probability sampling** is an ideal process where the probability of each animal of the target population to be included in the sample can be determined. As an example, in **simple random sampling**, such a probability is the same for all animals. In **nonprobability sampling**, the probability of each animal to be sampled varies (with some animals that may have no chance of being sampled) and such a probability cannot be exactly known. **Convenience sampling** of animals whose owner is available to participate into data collection, or of animals that are already included in data collection for reasons different from agents of vector borne agents, can be considered as nonprobability sampling. Valid estimates of sampling error, such as confidence intervals of prevalence of microbial agents in animals, cannot be obtained by convenience sampling. In practice, however, a judgment is made on the potential bias involved in sampling from animal populations. When no information is available on sampling probability, no inference should be attempted, and data only provide limited information. On the other hand, when the convenience sampling protocol is well defined, and good guesses on sampling probability can be made, confidence intervals of parameter estimates can be presented and interpreted with caution, keeping in mind sampling limitations.

Serial testing: the diagnostic process based on the execution of a first test (screening test) for an agent on all individuals of a sample, and the subsequent execution of a second test (confirmatory test) only on those individuals that were positive to the first test. Individuals resulting positive to both tests are

[6] Thrustfield M, 2006. Veterinary Epidemiology, 3rd edition, Blackwell Science Ltd. pp 624.

classified as positive. Serial testing increases specificity of the diagnosis, with the potential trade-off of decreasing sensitivity.

Stratified sampling: a sampling process where the units are classified into distinct groups, or strata, and a sample is then drawn from each stratum. Stratified sampling is usually carried out when strata are expected to differ in the parameter of interest and, in this case, it can provide more precise estimates (narrower confidence interval) than those that can be obtained by simple random sampling. For example, prevalence of an agent of a vector borne zoonosis may differ in different regions (or other administrative divisions) within a country (due to variations in habitat, animal populations, land use, etc.). Separate, stratified sampling can be carried out in each administrative division and results from the samples are combined to obtain a more precise prevalence estimate for the all country. Moreover, stratum specific estimates can be obtained by stratified sampling (see EFSA 2009[7] for further details).

Study population: the part of the target population from which a sample of units to be tested for an agent can be selected. This corresponds to the **sampling frame** or frame population. Animals selected for testing for other agents, or animals killed during hunting can be proposed as study populations to be sampled for testing on agents of vector borne zoonoses. If animals included in the study population are not a random sample of the target population, estimates of prevalence of vector borne agents may be affected by bias.

Sytematic error or bias: difference between estimates of an epidemiological parameter (i.e. prevalence of a vector borne agent) in the sample and in the target population, which is not attributable to a random selection of animals. In this report, bias might be caused by sampling animals from a study population that is not a random sample of the target population; as an example, by testing for antibodies against a vector borne agent a sample of blood sera collected from cattle within a control program of BHV1, the agent of infectious bovine rhinotracheitis (IBR), the prevalence of antibodies against a vector borne agent may be lower than that in the overall cattle population in a country, if herds tested for IBR belong to areas at a lower risk of exposure to vectors. Bias might also occur if the same number of units is sampled from geographic areas with a different population size within the same country. In this case, areas with large populations would be under represented, whereas areas with small populations would be over represented. As a consequence, if the risk of vector borne agents were greater in small population areas, prevalence estimates from this study population would be over estimated (EFSA 2009[7]).

Target population: the population about which a researcher or a competent authority wishes to draw conclusions by means of data collection. As an example, the prevalence of an agent is obtained in a sample of animals in order to draw conclusions about a defined animal population: the target population. In this report, target populations can be identified in the section "subpopulations to be covered" in the proposals for data collection on each agent and for each animal species.

[7] EFSA, 2009. Statistical analysis of temporal and spatial trends of zoonotic agents in animals and food Part I: Critical review of the statistical analysis carried out on the Community Summary Report 2006 data. The EFSA Journal 253, 1-77.

MATERIALS AND METHODS

INVENTORY OF AVAILABLE DATA

A narrative literature review was carried out to describe each agent's transmission cycle, with emphasis on the role of animals, and to gather information on the diagnosis of the infections in animals. *Ad hoc* questionnaires were used to obtain information on current and past data collection on vector borne zoonoses in animals in the EU, Norway and Switzerland, by national competent authorities. A systematic literature review of scientific publications was used to analyse data collection by research institutions in the same countries.

Narrative literature review

A comprehensive narrative literature review was carried out by experts for each agent to collect the necessary information to describe the agents' transmission cycle. Animal species involved in transmission were listed based on their role as reservoirs, or dead-end hosts. Sources of the agents for animals and humans were identified. These included vectors, the environment, and certain animal species. Clinical signs, antibody response, localization of the agents in animal tissues, and the possibility of detection by laboratory tests were also included in the narrative literature review.

Questionnaire for data collection in animals by competent authorities

A questionnaire, made up of eight Excel spreadsheets (one for each investigated vector-borne agent) was submitted to the representatives of the EU member states (MS) plus Norway and Switzerland. The questions were aimed at collecting information on:

The notifiable (reportable)/non notifiable status of the agent in animals;

The national reference laboratory for the agent or the disease;

Active and passive data collection on animals, official institution responsible for such activities, involved animal species, stage of sampling, time period, and diagnosis (details on questionnaires are reported in Annex A).

Systematic literature review of data collection in animals

Publications on data collections in animals were searched for in online data bases, including PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), CAB (http://www.cabi.org/), Web of Science (http://wok.mimas.ac.uk/), Science direct (http://www.sciencedirect.com/), Agris: (http://agris.fao.org/), Google Scholar (http://scholar.google.com/). Time span of publications to retrieve varied, for different agents, between 10 and 20 years (details on methods for literature search, including online data bases, and time span of publications, are given in Annex B). The main criterion to decide time span of the systematic literature search was the availability of publications for all the countries included in the study, meaning that, if no publication was found for certain countries, in the last ten years, the search was expanded backward to a 20 year limit. On the other hand, the ten year limit to the literature search for several agents was justified by recent developments in diagnostic techniques, which might make the comparison difficult between study results several years apart. Furthermore, for certain agents, information from publications over 10 year old had previously been summarised in comprehensive published reviews and these were used as information sources on data collection on animals.

From each publication, data were extracted on animal species and husbandry type, location of data collection, sampling design and stage, diagnosis (including details on laboratory techniques), occurrence of the agent. Data were entered in an *ad hoc* database built on Microsoft Access®, and, subsequently, imported in SAS® (http://support.sas.com/) and in the R software (www.r-project.org/) for data manipulation steps, statistical analysis, and graphical representation (including mapping).

More than one data collection activity was recorded from those publications reporting data obtained from different animal species or by using different approaches to sampling or diagnosis.

For each agent and animal species, logistic regression analysis was used to estimate prevalence and 95% confidence interval (95% CI) of positive results to diagnostic tests, by country, and by groups of countries. When several data collection activities on the same agent were recorded for the same animal species, non independence of results within the same data collection activity was taken into account by using Generalised Estimating Equation (GEE, GENMOD procedure, SAS®), yielding inflated standard error and producing wide 95% CI. In case of convergence problems by using GEE, a design-based approach was adopted using the SURVEYLOGISTIC procedure, with the CLUSTER statement, in SAS® (EFSA, 2009). When all tested animals were either negative or positive, the exact binomial 95% CI was obtained using the function *binom.test*, in R; in these cases, non independence of observations within the same data collection was not taken into account.

Tables synthesising results of the systematic literature review were automatically exported from R into Microsoft Word® by the *R2wd* package in R (http://cran.r-project.org/web/packages/R2wd/index.html). Further details on the data base and on data analysis, as well as country by county results are reported in Annex B.

Prevalence estimates were obtained to summarise the available information on the frequency of each agent by animal species, whereas 95% CI provided information on the uncertainty caused by the heterogeneity in the sources of data. The information gathered in this way was useful in defining the role of animals in transmission, in reviewing aspects of data collection and diagnostics, and served as as a basis for proposals for data collection (see below).

It is, however, to be specified that, based on prevalence estimates of vector borne agents that had been obtained from the systematic literature review, no valid inference can be made in animal species in entire countries or in groups of countries due to lack of harmonisation of data collection. In fact, results need to be cautiously interpreted as relative to the specific animal populations that were sampled, and to specific ecological and epidemiological situations (as defined by the objectives of research described in the reviewed publications).

Data from both the narrative and the systematic literature review were used to produce tables and maps of the geographic distributions of agents and vectors involved in transmission.

PROPOSALS FOR DATA COLLECTION ON VECTOR BORNE AGENTS IN ANIMALS

The main steps which are described below were followed to generate the proposals for the collection of data to be used in the analysis of temporal and spatial trends in zoonotic agents, and in the detection of the agents in previously free areas.

Creation of a list of animal species or groups

An initial list of animal species (or groups of species) as potential candidates for data collection was built based upon the above mentioned narrative and systematic literature reviews. Specific focus was on the distinction between those animal species playing a key role in the agent's maintenance and transmission (reservoir hosts), and those species that, although irrelevant for the agent's transmission, are nevertheless susceptible to infection and may suffer clinical or pathological consequences, as well as producing detectable antibody response (dead-end and accidental hosts).

Application of criteria for the selection of animal species or groups

Animal species were selected from the list created in the previous step by the application of criteria divided into five categories:

characterization of animal species (livestock, companion, wildlife), and role in transmission;

spatial distribution and land use;

response to infection and possibility of diagnosis;

practical factors affecting data collection;

current use of the species in disease monitoring programs in Europe.

Specific criteria were adapted to characteristics of each agent. Scores that were assigned through the application of criteria were summarised and carefully evaluated to reach a final list of animals to be included in options for data collection (active and/or passive) for each agent. Details on applied criteria and on the evaluation of scores are described in Annex C.

Specifications on data collection on selected animals species

Specifications on data collection on animal species identified in the previous steps were given as follows: subpopulations to be covered; agents and possible subtypes; sampling context and design, sampling stage; type of specimen to be taken and sampling technique; diagnostic methods to be used; recommendations for data submission.

A major distinction was made between active and passive data collections. Active data collection is based on sampling animals for the target population according to a pre established design, regardless of the occurrence of clinical signs. Passive data collection relies upon the reporting of suspect cases (based on the observation of defined clinical signs); the infection is subsequently to be confirmed by appropriate diagnostic tests. Factors associated with animal species and frequency of the agent were considered when deciding between active and passive data collections, and details are provided in specific sections. In depth information was collected in order to support proposals for data collection on each selected animal species, including response to infection (such as onset and duration of clinical signs and / or antibody response), comparative analysis of available diagnostic methods, factors affecting the risk of exposure to the agent, which can be used when specifying sampling design and inclusion criteria.

Dynamic models

Simple dynamic, mathematical models were built for a subset of agents, based on available information and including agents transmitted by ticks (*Borrelia burgdorferi* sensu lato), insects

(*Leishmania infantum*; a descriptive model was built for West Nile virus), or also by direct routes or the environment (*Francisella tularensis*).

These models were used to point out essential features of transmission and to explore the relationship between data collected on animals (i.e. prevalence of the agents or markers of infection, or incidence of clinical cases) and the intensity of transmission of the agents in the source of infection for animals and man (i.e. vectors or reservoir hosts). Key parameters (and gaps in knowledge) were identified, including rates of contact between infection sources and animals, antibody response and decay, incidence of clinical disease. In addition, the outputs of the dynamic models were used as support for sample size determination through the identification of epidemiologically meaningful trends in prevalence of antibodies against vector borne agents in animals and, therefore, worth being detected in data collection. For example, the output of the model performed for *B. burgdorferi* sl showed that increasing trends of host-seeking *Ixodes ricinus* ticks infected with *B. burgdorferi* sl resulted in an increasing trend of prevalence of specific antibodies in animals. Such a trend was characterised by an initial, slow increase of prevalence, followed by an intermediate phase of rapid increase, and by a final phase when a maximum prevalence value was reached. This output was used as a guide in numerical simulations for the determination of sample size needed to detect trends in prevalence of antibodies in animals. Details on dynamic models are given in Annex D.

Sampling design and size determination

Basic principles of sampling from populations are considered in the report, including the definition of target population, study population, probability and non probability sampling, sampling of non independent units, and stratification. Details on the application to data collection on vector borne agents in animals are described in Annex E.

Numerical simulations were carried out to determine necessary sample size to detect statistically significant trends. The number of years of data collection, and the trend worth being detected were based on indications that had been obtained through the dynamic modelling of the expected trend in the prevalence of antibodies against *B. burgdorferi* sl in animals, following an increasing trend in the abundance of infected host-seeking ticks. This was taken as a general example of the response of animals (antibody prevalence) to trends in the natural source of a vector borne agent (abundance of infectious ticks). Sample size was determined in different phases of increasing antibody prevalence (see Appendices D and E for details).

Significance level was fixed at 5%, and statistical power at 80%. Simulations were carried out by R. Programming codes are reported in Annex E. Results of sample size determination presented in Annex E are intended to be applied for active data collection on agents considered in this report, in those cases when trends in prevalence on animals (based on estimation of prevalence in different years) is identified as an objective for data collection. In general, trends in prevalence can be analysed for those agents that are known to be established in geographic areas and animal populations are exposed during subsequent years.

Detection of the agent in a geographic area is the objective of data collection when its occurrence is unknown, sporadic, or if it causes outbreaks in normally free populations. In these cases, sample size was established based on the minimum prevalence level to be detected (see Käsbohrer et al., 2010[8]).

[8] Käsbohrer A, Tenhagen BA, Fetsch A 2010. Development of harmonised survey methods for food-borne pathogens in foodstuffs in the European Union. Scientific report submitted to EFSA.

RESULTS

1. *BORRELIA*

Spirochetes of the genus *Borrelia* include the agents of Lyme borreliosis (*B. burgdorferi* sl group) and the agents of relapsing fevers. Lyme borreliosis is the most common vector borne disease of humans in temperate climates, therefore the data inventory and the proposals for data collection was focused on *B. burgdorferi* sl.

Relapsing fever borreliae are infrequent in Europe, and the role of animals in their transmission is uncertain. A narrative literature review on relapsing fever borreliae can be found in Annex G.

B. burgdorferi sl includes at least 19 genospecies with a worldwide distribution (EUCALB, 2011, Chu et al., 2008, Casjens et al., 2011). *B. burgdorferi sensu stricto* (ss), *B. garinii*, *B. afzelii*, *B. spielmanii*, *B. bavariensis*, *B. lusitaniae* (de Carvalho et al., 2010) are considered pathogenic to humans in Europe, while the pathogenicity of *Borrelia valaisiana* is uncertain. In humans, Lyme borreliosis is a multi-system disorder which can affect several tissues including skin, heart, nervous system, eyes, kidneys and liver (Gratz, 2006).

1.1. Inventory of available data

1.1.1. Transmission cycle of *B. burgdorferi* sl

In Europe, spirochetes belonging to the *B. burgdorferi* sl group are transmitted in natural cycles between the tick *Ixodes ricinus* and vertebrate reservoir hosts (Gern and Humair, 2002; Mannelli et al., 2011). *I. persulcatus* is also involved in north eastern Europe. Clinical consequences of the infection may take place in humans and in other animal species that, normally, do not play a key role in transmission, such as dead-end hosts.

Reservoir hosts, that are infected with *B. burgdorferi* sl by feeding ticks, and that are, subsequently, capable of transmitting the infection to other susceptible ticks, include small and medium size mammals, such as wood mouse (*Apodemus sylvaticus*), yellownecked field mouse (*Apodemus flavicollis*), black striped mouse (*Apodemus agrarius*), bank vole (*Myodes glareolus*), meadow vole (*Microtus agrestis*), edible dormouse (*Glis glis*), hazel dormouse (*Muscardinus avellanarius*), garden dormhouse (*Eliomys quercinus*), rats (*Rattus norvegicus, Rattus rattus*), squirrels (*Sciurus vulgaris*, *Sciurus carolinensis*, *Tamias* spp.), hares (*Lepus europaeus, Lepus timidus*), shrews (*Neomys fodiens, Sorex minutus, Sorex araneus*) and European hedgehog (*Erinaceus europaeus*) (Derdakova and Lencakova, 2005; Matuschka et al., 1994; Richter et al., 2004a; Richter et al., 2004b; Richter et al., 2011; Vourc'h et al., 2007). Among birds, several species of passerine birds and *Galliformes* are reservoir hosts, including black birds and thrushes (*Turdus merula, T. philomelos*), nightingale (*Luscinia megarhynchos*), wren (*Troglodytes troglodytes*), pheasants (*Phasianus colchicus*) (Craine et al., 1997; Hanincová et al., 2003; Mannelli et al., 2005). Some reptiles (*Lacerta viridis, Lacerta agilis, Podarcis tauricus, Podarcis muralis*) have shown to play a role as reservoirs of *B. burgdorferi* sl (Richter and Matuschka, 2006; Amore et al., 2007; Földvári et al., 2009). Each animal species may serve as a reservoir host for only certain *B. burgdorferi* sl genospecies, although such a genospecies specificity is not absolute (Gern, 2008, see also section 1.1.4.).

The reservoir role of carnivorous species, such as foxes (*Vulpes vulpes*) and badgers (*Meles meles*) is probably limited (Gern, 2008; Gern and Sell, 2009). Domestic dogs are susceptible to the infection by *B. burgdorferi* sl and may suffer clinical disease and develop a antibody response (see below). In experimental studies in the USA, dogs were shown as reservoirs for a short time after infection, but

their significance in depositing fed ticks in the environment is probably very limited (Grauer et al., 1998).

Wild and domestic ungulates, such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), wild boar (*Sus scrofa*), cattle (*Bos taurus*) and sheep (*Ovis ovis*) are important hosts for adult ticks. They are, however, considered as incompetent as reservoirs for *B. burgdorferi* sl (dead-end hosts) even though *B. burgdorferi* sl may pass among ticks feeding in close proximity on the skin of these specie (non systemic transmission, or via co-feeding ticks) (Odgen et al., 1997).

Horses may suffer clinical consequences following the infection by *B. burgdorferi* sl, but the role of this speces in transmission is generally negligible.

A diagram was drawn in order to visualize the transmission cycle of *B. burgdorferi* sl and the role of animals (Figure 1). Here, arrows represent transmission of spirochetes, whereas animals are divided into three main categories: reservoir hosts, acquiring the infection from infected ticks, and passing it to susceptible ticks (as shown by the two arrows); ungulates, that may be exposed to *B. burgdorferi* sl, but do not, generally, infect susceptible ticks (only one, unidirectional, arrow); dogs, that might infect ticks (dashed arrow), but with no role in transmssion. Man may acquire the infection from ticks and develop clinical disease, and can be considered a dead-end host (unidirectional arrow). This schematic representation will be used as a starting point for the selection of animal species for data collection (see section 1.3.).

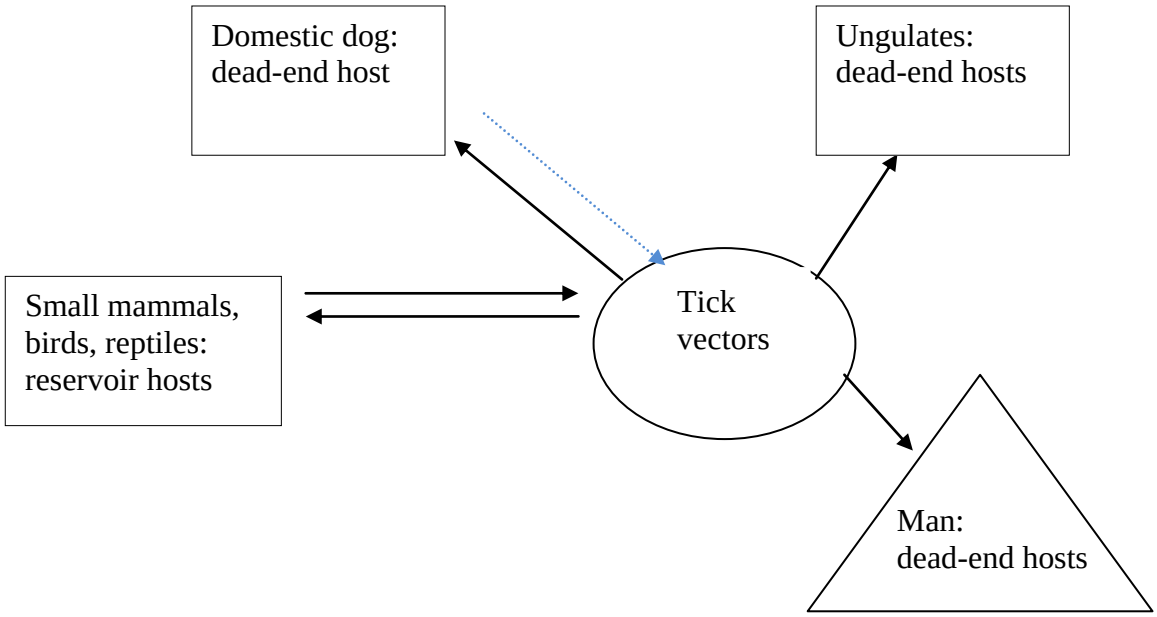

Figure 1. Diagram of the transmission of *B. burgdorferi* sl with specific focus on the role of animals.

1.1.2. Diagnosis of *B. burgdorferi* sl in animals.

Clinical signs in animals caused by B. burgdorferi sl.

The infection by *B. burgdorferi* sl in animals may be asymptomatic or with generic signs, such as fever, dullness, and anorexia. Articular forms are characterized by lameness and stiffness, articular swelling and pain. Behavioural changes are reported in horses (see specific sections for each animal species).

Laboratory diagnosis of B. burgdorferi sl in animals

Laboratory diagnosis of *B. burgdorferi* sl infections in animals, could be conducted either by direct or indirect methods.

Direct laboratory diagnosis

Amplification of *B. burgdorferi* sl DNA fragments by polymerase chain reaction (PCR) is the most sensitive techniques for the direct diagnosis of the infection in animals. Targets for PCR include gene fragments encoding for 16S rRNA, Fla gene, OspA gene, 5s-23s intergenic spacer region. PCR positive samples can subsequently be characterized by genospecies using DNA sequencing, reverse line blotting (RLB), repeated fragment length polymorphism (RFLP). In animals, PCR can be carried out on blood, skin, ear, and tail biospsies, oropharyngeal and cloacal swabs (in birds). Kidney, heart, bladder (and urine) can be used in dead animals. Culture of spirochetes has also been used, but slow and delicate bacterial growth reduces its use in practice (Kybicová et al., 2009; Zygner et al., 2008; Welc-Faleciak et al., 2009).

Indirect laboratory diagnosis

Enzyme linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), western blotting (WB), indirect hemoagglutination test (IHA) can be used to detect antibodies (IgG and/or IgM) against *B. burgdorferi* sl in animals. Reduced specificity of tests can be due to cross reactions with antibodies against a wide variety of other microbial agents (Pejchalová et al., 2006). Serial testing, where animals positive to ELISA or IFA are subsequently tested by a confirmatory test such as WB, is used to increase specificity of the diagnosis.

Recently, a synthetic peptide that reproduces the sequence of an immunodominant, conserved region (designated the sixth invariable region, or IR6) of a *B. burgdorferi* sl surface lipoprotein named Vls-E, has been used to develop serological tests for humans and animals (Chandrashekar et al., 2010; Johnson et al., 2008). The IR6 is both structurally and antigenically conserved among pathogenic *B. burgdorferi* sl strains and genospecies. All animals that were experimentally infected, including mice, monkeys and dogs, produced strong antibody response to the IR6 sequence. The peptide is considered to be a marker for active infection (Liang et al., 2000). Consequently, vaccinated animals test negative. A ELISA test (C6 ELISA) was developed based on the IR6 peptide. A quantitative C6 ELISA, was also designated to measure the level of C6-specific antibody, in antibody-positive animals. Research demonstrated that the C6 antibody declines after effective treatment and determining baseline and 6 months C6 antibody levels may be useful to monitor antibody levels on treated dogs (Levy et al., 2008).

1.1.3. Data collection on *B. burgdorferi* sl in animals by competent authorities of EU countries, Norway and Switzerland.

In this section, past and current data collection on *B. burgdorferi* sl in animals is EU countries, Norway and Switzerland are summarised, based upon information gathered through the questionnaire submitted to members of the Task Force on Zoonoses data collection.

Summary of results of questionnaires to the Task Force on zoonoses data collection

Eleven MSs (Denmark, Estonia, Finland, France, Hungary, Latvia, Portugal, Romania, Slovakia, Slovenia, Sweden), plus Norway and Switzerland responded to the questionnaire on data collection on *B. burgdorferi* sl.

B. burgdorferi sl is notifiable in animals in Slovenia only. Past or current data collection on *B. burgdorferi* sl in animals was reported in Finland, France, Hungary, Norway, Slovakia, Slovenia, Sweden.

Country by country results

Main information on data collection activities on *B. burgdorferi* sl carried out in the countries that replied to the questionnaires is summarised in Table 1. Country by country results are reported in Annex A.

Table 1. Summary of results of questionnaire on data collection on *B. burgdorferi* sl in animals by competent authorities in seven countries that reported past or current activities[(a)].

Country	Active data collection			Passive surveillance			National Reference Laboratory
	Time span	*Animal species*	*Diagnosis*	*Time span*	*Animal species*	*Diagnosis*	
Finland	2000 – present				Dog, horse	IFA	Finnish Food Safety Authority
France		Dog, fox, *Tamias sibiricus*	Serology, PCR, bacterial culture				Pasteur Institute
Hungary					Dog, horse	CFT	Central Agricultural Office
Norway					Dog, horse	IFA	
Slovakia					Dog	ELISA	
Slovenia					Dog	IFA	
Sweden	2001	Wild birds	PCR		Dog	IFA	

(a) No data collection activities on *B. burgdorferi* sl in animals, nor a reference laboratory, were reported in the questionnaires from Denmark, Estonia, Romania and Switzerland.

1.1.4. Systematic literature review on data collection on *B. burgdorferi* sl in animals

The literature search was carried out on publications from 2000 to 2010. This time span was justified by the recent developments of diagnostic tools, such as accurate serological tests, and the availability of comprehensive narrative review of publications carried out before 2000 (see, for example, EUCALB).

Results of active data collection on B. burgdorferi sl by animal species

Fifty eight publications, from 19 countries (Austria, Bulgaria, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Norway, Poland, Portugal, Slovakia, Spain, Sweden, Switzerland, United Kingdom) were retrieved on active data collection on animals.
The main results of active data collection are summarised in Table 2. Results by country, including information on diagnostic method and sampling design, as well as specific bibliographic references are presented in Annex B.

Prevalence estimates vary greatly among animal species but, in general, indicate a relatively frequent exposure to *B. burgdorferi* sl in the countries considered in the systematic literature review. These results cannot, however, be considered as representative estimates for each animal species, and the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study.

Data on small mammals (including rodents, insectivores, squirrels) and dogs were collected from a relatively great number of countries. The prevalence estimates obtained for reservoir hosts are mostly associated with infection by specific genospecies of *B. burgdorferi* sl: *B. afzelii* and *B. burgdorferi* ss for small mammals, *B. garinii* and *B. valaisiana* for birds, and *B. lusitaniae* for lizards, although such a genospecies – specificity is not absolute.

In comparison with results on other species, the prevalence estimate on dogs, and the associated confidence interval, might be characterised by an acceptable degree of representivity, due to the number of considered studies and the recent development of accurate indirect laboratory tests for this species.

A relatively great number of horses and cattle were tested, however, in a limited number of studies. As a consequence, prevalence estimates obtained on these species are representative of only a limited number of locations or specific epidemiological situations.

It is to be noted that, in domestic and wild ungulates, antibodies were usually detected by indirect laboratory tests which, in certain cases, might yield false positive results. As an example, greatest prevalence (40.4%) was reported in chamois that were tested in a single study by IFA antibody detection. As a consequence, results presented in these synthetic tables should be interpreted with caution. Further details can be found in Annex B, on the results of the systematic literature review by country.

The finding of *B. burgdorferi* sl in a limited sample of badgers (*Meles meles*) is of interest since it was achieved by isolation of the agent in culture. Moreover, badgers reach a relatively high population density in certain European countries (Gern and Sell, 2009).

Table 2. Results of active data collection on *B. burgdorferi* sl in animal species, based on the systematic review of publications in the EU, Norway and Switzerland, from 2000 through 2010[(a)].

Animal species	N of countries	N of collection activities	N of tested animals	N of positive animals	% Prevalence	Lower 95% CL	Upper 95% CL
Small mammals[(b)]	12	60	4220	751	17.80	13.28	23.43
Dog[(c)]	10	23	5796	560	9.66	5.64	16.07
Birds[(d)]	6	28	1706	229	13.42	6.53	25.60
Lizard[(e)]	4	6	427	38	8.90	4.06	18.40
Horse[(f)]	4	4	3211	688	21.43	14.74	30.07
Deer[(g)]	3	4	451	129	28.60	15.29	47.08
Cattle[(h)]	3	3	1285	346	26.93	14.28	44.90
Sheep and Goats[(i)]	1	3	297	57	19.19	14.12	25.55
Wild boar[(j)]	1	1	642	82	12.77	10.41	15.58
Chamois[(k)]	1	1	114	46	40.35	31.76	49.58
Fox[(l)]	1	1	84	7	8.33	4.02	16.46
Badger[(m)]	1	1	8	3	37.50	12.54	71.52

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study; (b) Amore et al., 2007, Barandika et al., 2007, Christova and Gladnishka, 2005, de Carvalho et al., 2010, Gil et al., 2005, Gray et al., 2000, Huegli et al., 2002, Khanakah et al., 2006, Kybicová et al., 2008, Michalik et al., 2005, Pawelczyk et al., 2000, Pawelczyk et al., 2004, Rizzoli et al., 2004, Skuballa et al., 2007, Stefancíková et al., 2004, Stefancíková et al., 2008, Vostal et al., 2003, Vourc'h et al., 2007. (c) Amusategui et al., 2008, Bhide et al., 2004, Couto et al., 2010, Egenvall et al., 2000, Gerber et al., 2007, Goossens et al., 2001, Jäderlund et al., 2007, Jensen et al., 2003, Kybicová et al., 2009, Menn et al., 2010, Merino et al., 2000, Pantchev et al., 2009, Pejchalová et al., 2006, Skotarczak et al., 2003, Skotarczak et al., 2005, Sobrino et al., 2008, Solano-Gallego et al., 2006, Speck et al., 2007, Welc-Faleciak et al., 2009, Zygner et al., 2009 (d) Amore et al., 2007, Gronesova et al., 2008, Gryczyńska et al., 2004, Kaiser et al., 2002, Michalik et al., 2008, Schwarzová et al., 2006, Staszewski et al., 2008 (e) Amore et al., 2007, Földvári et al., 2009, Majláthová et al., 2006, Majláthová et al., 2008; (f) Egenvall et al., 2001, Hansen et al., 2010, Maurizi et al., 2010, Stefancíková et al., 2008; (g) Bhide et al., 2004, Pichon et al., 2000, Skarphédinsson et al., 2005; (h) Lengauer et al., 2006, Stefancíková et al., 2002, Stefancíková et al., 2008; (i) Bhide et al., 2004, Trávnicek et al., 2002; (j) Juricová and Hubálek, 2009; (k) Ortuno et al., 2003; (l) Sobrino et al., 2008; (m) Gern and Sell, 2009.

Results of passive data collection on B. burgdorferi s.l. by animal species

Passive data collection on *B. burgdorferi* sl in animals is summarised in Table 3. Further information and specific bibliographic references are reported in Annex B.

Retrieved publications are from four countries and mostly describe case reports in dogs. In two reports, cattle and cats were considered. The proportion of suspect cases which were, subsequently, confirmed by laboratory tests varied across studies. This might be due to the aspecific clinical signs of Lyme borreliosis in animals (leading to the possible inclusion of animals affected by other diseases), to variations in criteria for suspect case definition, and to differences in laboratory test accuracy.

Table 3. Results of passive data collection on *Borrelia burgdorferi* sl in animal species based on the systematic review of publications in the EU, Norway and Switzerland, from 2000 through 2010.

Animal species	Country (reference)	Criteria per suspected case definition	N suspected	N confirmed	Genospecies identified	Test procedure	Confirmation test	Tissue type
Dog	Poland (Wodecka et al., 2009)	Arthritis (swollen joints and enlarged prescapular lymph nodes); malaise (fever, loss of appetite, and fatigue) and lameness; myocarditis, a renal signs, neurological dysfunction.	18	7	*B.burgdorferi s.s*	Single	PCR	blood
Dog	Sweden (Jäderlund et al., 2009)	Exposure to ticks, fever and lameness, primary inflammatory diseases of the central nervous system (CNS), neurological signs.	54	4	-	Parallel	PCR and IFA	serum
Dog	Sweden (Jäderlund et al., 2009)	Exposure to ticks, fever and lameness, primary inflammatory diseases of the central nervous system (CNS), neurological signs.	54	0	-	Parallel	PCR and IFA	CSF
Dog	Switzerland (Speck et al., 2007)	History of tick infestation, lameness, neurological signs, nephropathy, lethargy, anorexia, fever	98	21	-	Serial	ELISA, WB	serum
Dog	Switzerland (Speck et al., 2002)	History of tick infestation, lethargy, anorexia, fever, lameness, neurological signs, nephropathy	98	1	*B.afzelii*	Serial	Culture, PCR	blood, urine, SF, CSF, skin biopsy
Dog	United Kingdom (Shaw et al., 2005)	Recurrent pyrexia, acute-onset pyrexia with weakness and lethargy, anaemia and/or thrombocytopenia, polyarthritis and/or muscle pain, splenomegaly and/or lymphadenopathy, intraocular inflammation with systemic signs.	120	5	-	Single	PCR	Blood
Cat	United Kingdom (Shaw et al., 2005)	Recurrent pyrexia, acute-onset pyrexia with weakness and lethargy, anaemia and/or thrombocytopenia, polyarthritis and/or muscle pain, splenomegaly and/or lymphadenopathy, intraocular inflammation with systemic signs.	60	2	-	Single	PCR	blood
Cattle	Switzerland (Lischer et al., 2000)	Fever, stiffness and swollen joints, reduced milk production, chronic weight loss, laminitis, abortion, possible exposure to ticks in endemic areas.	2	2	*B.burgdorferi s.s., B.afzelii*	Single	real-time PCR	synovial fluid, urine, serum, buffy coat, milk, cerebrospinal fluid

1.1.5. Information on the occurrence and geographical distribution of *B. burgdorferi* sl, host and vectors

Agent

B. burgdorferi sl is widespread across the geographic range of tick vectors (*I. ricinus* and *I. persulcatus* in Europe) and it is potentially present in deciduous woods below 1000 m above sea level. Moreover, recent findings suggest expansion of vectors and spirochetes at greater altitudes (Daniel et al., 2009).

Among the genospecies belonging to the *B. burgdorferi* sl group, *B. garinii* and *B. valaisiana* are found across much of Eurasia, *B. afzelii* is less abundant in the British Isles compared with continental Eurasia, *B. burgdorferi* ss is very common in Western Europe, while *B. lusitaniae* is typical of Southern Europe and the Mediterranean area (Kurtenbach et al., 2006, Amore et al., 2007).

Few countries in Europe have made Lyme borreliosis a compulsory notifiable disease in humans; therefore it is possible to make only approximate estimates of incidence (Stanek et al., 2011). A gradient of increasing incidence from West to East Europe is present with the highest incidence in Central-Eastern Europe (Gratz, 2006).

In the systematic literature review on data collection in animals, positive results were reported from all countries where data collection was carried out (see section 1.1.4).

The geographic distribution of the reported genospecies in our systematic literature review is shown in Table 4. Results of data collection activities where genspecies characterisation was carried out are reported.

It must be taken into account that findings were affected by different species of animal hosts that were examined in each country. As an example, since in Italy *B. burgdorferi* sl was only found and characterised in lizards (in publications in the time span considered in this review), *B. lusitaniae* (typically associated with lizards) was identified (Amore et al., 2007), and rodent-associated genospecies such as *B. afzelii* and *B. burgdorferi* ss were not reported in Table 4.

Furthermore, only the detection of agents in animal tissues is reported in Table 4, whereas several ecological studies are based on testing of ticks collected from animals. As an example, *B. burgdorferi* ss was found in blood from a single bird in Poland (Michalik et al., 2008, as reported in Table 4), but engorging ticks collected from birds in the same study were infected by bird-associated genspecies such as *B. garinii* and *B. valaisiana* (not reported in Table 4).

Based on the aforementioned, data shown in Table 4 cannot be considered as estimates of the spatial distribution of genospecies, however, they must be interpreted as a summary of recent studies on animals in Europe.

These results confirm that the host association of *B. burgdorferi* sl genspecies is not absolute. In fact, *B. garinii* (that is normally associated with birds) was repeatedly found in small mammals (including hedgehogs in Germany). It must, however, be pointed out that, in small mammals, the prevalence of *B. afzelii* and *B. burgdorferi* ss was much greater than the prevalence of *B. garinii*, therefore confirming previously known host preferences of *B. burgdorferi* sl genospecies (data on the prevalence of each genspecies are not presented here).

The detection of *B. afzelii* and *B. garinii* confirms the susceptibility of dogs to these pathogenic genospecies. The detection of live *B. burgdorferi* sl, belonging to three genospecies, in the skin of deer tested in France (Pichon et al., 2000) contributes to the controversial issue of the role of ungulates in the transmission of these agents (which we, nevertheless, classify among dead-end hosts). Three *B. burgdorferi* sl genospecies (*B. afzelii*, *B. garinii*, and *B. spielmanii*) were identified in European hedgehogs (*Erinaceus europaeus*) in Germany (Skuballa et al., 2007). It is worth noting that two

species of dormouse, the garden dormouse (*Eliomys quercinus*) and the hazel dormouse (*Muscardinus avellanarius*) are considered the main reservoir hosts for *B. spielmanii* (Richter et al., 2004b; Richter et al., 2011).

Table 4. Distribution of *B. burgdorferi* sl genospecies in animals, by active data collection, based on systematic literature review from 2000 through 2010[(a)].

Country	Animal species	*B. afzelii*	*B. burgdorferi* ss	*B. garinii*	*B. lusitaniae*	*B. spielmanii*	*B. valaisiana*
Czech Republic	Dog[(b)]	1[(c)]	0	1	0	0	0
	Small mammals[(d)]	1	1	1	0	0	0
France	Deer[(e)]	1	1	1	0	0	0
	Small mammals[(f)]	1	1	0	0	0	0
Germany	Small mammals[(g)]	1	0	1	0	1	0
Hungary	Lizard[(h)]	0	0	0	1	0	0
Ireland	Small mammals[(i)]	1	0	1	0	0	0
Italy	Lizard[(j)]	0	0	0	1	0	0
Poland	Birds[(k)]	0	1	0	0	0	0
	Dog[(l)]	1	0	0	0	0	0
	Small mammals[(m)]	0	1	0	0	0	0
Portugal	Small mammals[(n)]	0	0	0	1	0	0
Slovakia	Birds[(o)]	0	1	1	0	0	0
	Lizard[(p)]	1	0	0	1	0	0
	Small mammals[(q)]	1	0	0	0	0	0
Spain	Small mammals[(r)]	1	1	0	0	0	0
Switzerland	Badger[(s)]	1	0	0	0	0	1
	Small mammals[(t)]	1	0	1	0	0	0

(a) Findings presented in this table were affected by different species of animal hosts that were examined in each country and, therefore, cannot be considered as valid estimates of the geographic distribution of genospecies; (b) Kybicová et al., 2009, Pejchalová et al., 2006; (c) 1: the genospecies was found in animal tissues, 0: the genspecies was not found; (d) Kybicová et al., 2008, (e) Pichon et al., 2000; (f) Vourc'h et al., 2007; (g) Skuballa et al., 2007; (h) Földvári et al., 2009; (i) Gray et al., 2000; (j) Amore et al., 2007; (k) Michalik et al., 2008; (l) Zygner et al., 2009; (m) Michalik et al., 2005; (n) de Carvalho et al., 2010; (o) Gronesova et al., 2008, Schwarzová et al., 2006; (p) Majláthová et al., 2006; (q) Stefancíková et al., 2008; (r) Barandika et al., 2007; (s) Gern and Sell, 2009; (t) Huegli et al., 2002.

Hosts

The presence of the most relevant sylvatic host species for *B. burgdorferi* s.l. in EU MSs, Norway and Switzerland is presented in Annex F.

Vectors

The geographical distribution of *I. ricinus* is reported in Figure 2.

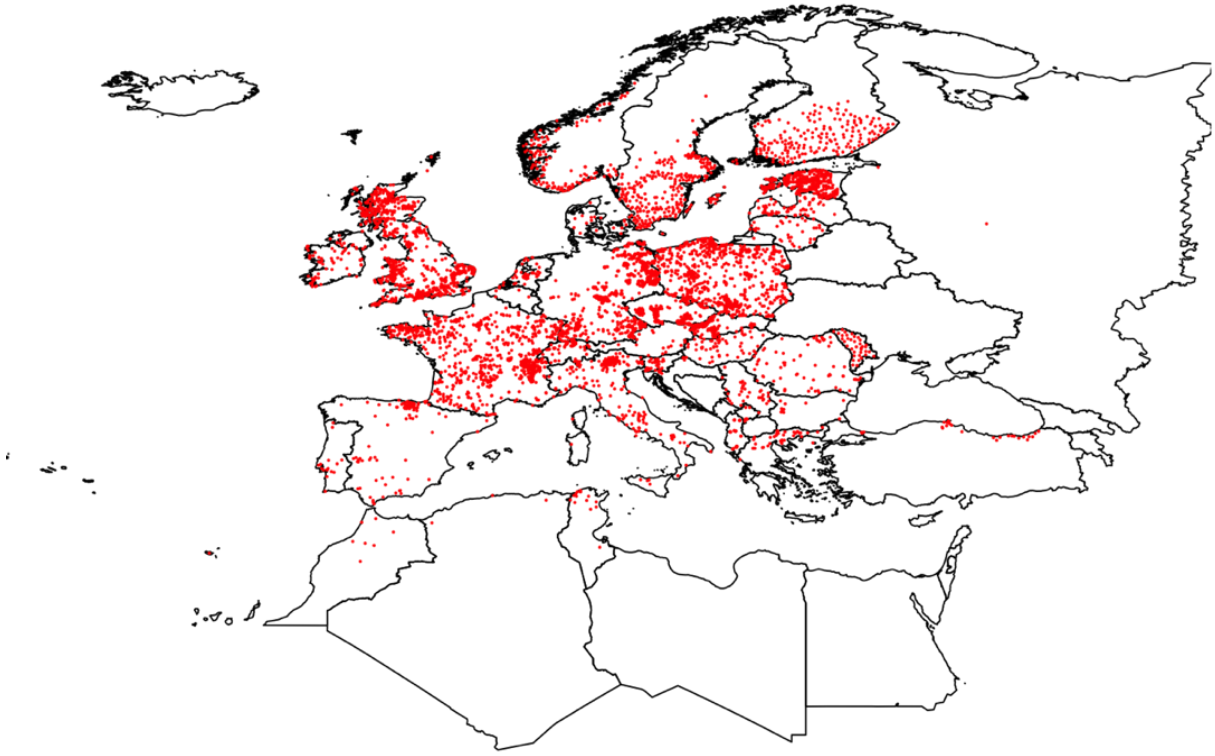

Figure 2. Distribution of *Ixodes ricinus* in Europe. Dots indicate locations where the tick species was observed (based on EFSA, 2010).

Proposal for data collection on *B. burgdorferi* sl in animals

The application of criteria for the selection of animal species to be proposed for data collection on *B. burgdorferi* sl led to the general conclusions described below. Details on the criteria applied are illustrated in Annex C.

The following animal species were included in proposals for data collection: dog (active and passive data collection), horses and domestic ruminants (active data collection).

Wildlife competent reservoir hosts and wild ungulate, although used in data collection for research purposes, are not included in proposals due to drawbacks summarised below.

Wildlife competent reservoir hosts (small mammals, birds, reptiles) are characterised by a certain degree of genospecies specificity. Consequently, in order to study the occurrence and trends of the pathogenic genspecies belonging to *B. burgdorferi* sl, data collection should be simultaneously carried out on several species of wild animals. Such activities would be practically difficult, labour intensive, and costly.

Wild ungulates (deer, wild bovidae and wild boar) serve as hosts for tick vectors and are exposed to *B. burgdorferi* sl. Moreover, the prevalence of antibodies can reach relatively high levels (Table 2). On the other hand, these species are less available for harmonised sampling in comparison with domestic animals. Therefore, although data collection on wild ungulates can be useful at the local level, it is not included in proposals within this report.

A summary of proposals of data collection on *B. burgdorferi* sl in animals is presented in Table 5.

1.1.6. Data collection on *B. burgdorferi* sl in dogs

Dogs were frequently used animals in research studies and as indicators of *B. burgdorferi* sl in Europe and in North America. Advantages of the dog over other species include widespread distribution and close association with humans, with whom dogs may share exposure to outdoor environments and ticks. Furthermore, dogs are more prone to be exposed to tick bites compared to people frequenting the same habitats (Hamer et al., 2009).

Specification on active data collection on B. burgdorferi sl in dogs.
Subpopulations to be covered

Two options regarding the type of dogs can be envisioned: privately-owned dogs and shelter dogs. Information can be collected, based upon owners' compliance, on exposure to ticks, place of origin, exposure to wooded habitat (favourable to *I. ricinus* and *B. burgdorferi* sl), and treatments that might interfere with antibody response. The obvious limit to the use of shelter dogs is the lack of the aforementioned information. On the other hands, shelter dogs are easily accessed for sampling.

Sampling should only include dogs frequenting outdoor habitats, below 1000 m above sea level. In fact, although *B. burgdorferi* sl is currently expanding its range above such an altitude in certain countries, establishing an altitudinal limit for sampling would improve harmonisation of data collection.

Dogs which are regularly treated with repellents including collars and spot-on repellents, and dogs treated with antibiotics or corticosteroids in the last month before sampling should be excluded. Alternatively, the inclusion of dogs treated with tick repellents can be considered if the information on treatment is provided. This would allow an evaluation of the effect of treatment and a proper adjustment of prevalence estimates.

Agents and possible subtypes

Infection by *B. burgdorferi* sl, with no special focus on specific genospecies is recommended, to optimise usefulness of data collection from dogs (and other animals).

Sampling context and design, sampling stage

Privately owned dogs should be sampled in the context of visits by veterinarians. Given the difficulties in the realisation of a formal random sampling of owned dogs, convenience sampling is to be adopted, based on availability by owners and veterinarians, as well as compliance with inclusion criteria described above.

Serum samples are easily collected from dogs that can also be routinely sampled and tested for other agents (including vector-borne agents). Information on exposure to habitats and movements should be gathered by interviewing the owners.

Shelter dogs can be more easily accessed by competent authorities. However, information on habitat frequentation and on the use of tick repellent and antibiotics might not be available.

In each country, sampling design should be stratified at least at the level of first order administrative divisions, or at the level of second order administrative divisions, if possible. In practice, once a country-level sample size is determined, dogs should be separately sampled from each administrative area. This would increase precision of prevalence estimates at the country level, and would allow analysis of trends at a relatively small scale (EFSA 2009, 2011). Information on the canine population in each administrative division would be useful for the application of weights in the calculation of the country-level prevalence. If the sample size in each administrative division were proportionate to the canine population, weighting would not be necessary. However, proportionate stratified sampling

would result in very small samples from areas with small canine population. Consequently, such an option should be considered on a case by case basis (see Annex E for details on stratified sampling).

Type of specimen and sampling technique

For active data collection on dogs, the collection of sera is recommended, for the subsequent search of antibodies against *B. burgdorferi* sl.

Diagnostic methods

Commercial, indirect diagnostic tests which are specific for dogs can be used. Antibodies against *B. burgdorferi* sl IR6 antigen are detectable even as early as three weeks after infection and a strong response is maintained for at least 69 weeks (Liang et al., 2000, Straubinger, 2000). By using a quantitative ELISA, Levy et al. (2008) showed that antibody levels in dogs declined 12.9% after 12 months from infection. Seroconversion may occur prior to, or in the absence of clinical signs. Tests based on the new C6 peptide yields positive results only during active infection by *B. burgdorferi* sl. Therefore, these tests do not give false positive results following cross-reaction with other spirochetes (e.g. *Leptospira*) or with vaccine-induced antibodies (Liang et al., 2000). In conclusion, a test is recommended based on the detection of antibody to the sixth invariable region (IR 6) of the VlsE outer membrane lipoprotein, a 25-amino-acid peptide termed C6. Sensitivity and specificity are estimated as 95.5% and 100%, respectively (Chandrashekar et al., 2010; Johnson et al., 2008; Levy et al. 2008).

Results of tests providing qualitative outcomes (positive or negative) can be directly interpreted to classify dogs as positive or negative, whereas when using tests providing quantitative outcomes (such as absorbance in ELISA) dogs are to be considered as positive when the corresponding test outcome exceeds the cut – off value (that can be established by each test specific procedure).

Data submission

Data submitted to EFSA, preferably separate by first order administrative division, should include the number of tested and of positive individuals, as well as the canine population if available. Privately owned dogs and shelter dogs (if considered) should be clearly distinguished. The effects of geographic location and of dog type could in this way be considered at the level of trend analysis, to obtain comparable estimates of trends. If dogs treated with tick repellent are included in sampling, information on treatment should be provided.

Specification on passive data collection on B. burgdorferi sl in dogs.

The usefulness of passive surveillance of *B. burgdorferi* sl in dogs to study trends of the agent is reduced by the great variability of clinical signs in dogs. Furthermore, the occurrence of clinical signs in dogs infected by *B. burgdorferi* sl is inconstant and unrelated with antibody response (Greene et al., 2006). In spite of these problems, and considering that passive data collection in dogs is currently carried out in some European countries (see section 1.3.1.) we suggest recording the number of suspect cases and the number of confirmed cases, as indexes of the presence of the agent in a geographic area. Obviously, the interpretation of these results will be cautious.

Subpopulations to be covered

All dogs presenting clinical signs which can be caused by *B. burgdorferi* sl infection can be considered as suspect cases (see below), excluding those individuals that have never frequented an outdoor environment and, therefore, are unlikely to have had any contact with tick vectors.

Agents and possible subtypes

All genospecies belonging to the *B. burgdorferi* sl group are to be considered.

Sampling context and design, sampling stage

In the case of passive data collection, suspect cases should be reported, to competent authorities by private veterinarians and a laboratory confirmation should be carried out.

Definition of suspect cases should be based on the most common signs of infection. Dogs infected by *B. burgdorferi* sl can show fever, apathy, arthritis, kidney damage, meningitis, encephalitis, neuritis and myocarditis. However, infected dogs may remain asymptomatic even in the presence of an antibody response (Speck et al., 2007). Clinical signs to classify suspect cases will, therefore, include lameness, often with swelling of limb joints.

Type of specimen and diagnostic tests

Laboratory confirmation of suspect cases should be carried out by PCR on blood (during early infection) and synovial fluid (in the presence of articular sings). The use of indirect diagnosis is not recommended since a positive serological test could be associated with a past infection, and unrelated with current clinical signs. PCR can be used for direct detection of *B. burgdorferi* sl in dogs, mainly on blood, but also skin, cerebrospinal fluid (CSF), joints, and urine. Spirochetemia is temporary and, therefore, detection is possible during a short period in the early stage of infection, and this must be kept in mind while considering PCR for the diagnosis of *B. burgdorferi* sl in dogs (Bauerfeind et al., 1998; Straubinger, 2000; Speck et al., 2007; Wodecka et al., 2009). The presence of the detected sequences does not necessarily constitute a proof of disease, because the PCR does not allow discrimination between DNA of a live or a dead organism (Wodecka et al., 2009).

Data submission

Only dogs that were positive by PCR should be considered as confirmed cases of infection by *B. burgdorferi* sl. Reporting should include both the number of suspect cases that are subsequently tested for confirmation, and the number of confirmed cases, at the level of first administrative areas.

1.1.7. Data collection on *B. burgdorferi* sl in horses

Horses are susceptible to *B.burgdorferi* sl and may suffer clinical signs. Harmonised sampling from horses is possible. However, geographic distribution of horses and frequentation of habitats favourable to tick vectors might be very heterogeneous across Europe. Clinical signs in horses are very generic and unrelated with antibody response. Most of the infections are subclinical. Arthritis and uveitis are reported (Maurizi et al., 2010). Given the variability of clinical signs and of clinical diagnostic criteria of Lyme borreliosis in horses, passive data collection would be very difficult to harmonise. As a consequence, only active data collection is proposed for horses.

Specification on active data collection on B. burgdorferi sl in horses.

Subpopulations to be covered

Sampling should include horses frequenting outdoor habitats,. Horses that are regularly taken outdoor (below 1000 m above sea level) both companion and farm horses, should be included in sampling. Horses used only in corral or non natural competition settings are excluded since exposure to tick vectors for *B. burgdorferi* sl is unlikely.

Agents and possible subtypes

All genospecies belonging to the *B. burgdorferi* sl are to be considered.

Sampling context and design, sampling stage

Horses can be subject to random sampling, given the existence of anagraphic lists. Additionally, blood serum can be collected from horses from other disease control plans, such as equine infectious anaemia or West Nile virus. Under these circumstances, the same horses can be sampled for *B. burgdorferi* sl.

When sampling horses that are grouped into farms, farms would be first seleced (primary sampling units), whereas individual horses would be selected within each farm, in a multistage sampling design. Non independence of observations within the same farms should be taken into account when determining sample size and when analysing the data (see Annex E for details on sampling).

Type of specimen and sampling technique

Blood serum can be taken for subsequent antibody detection tests.

Diagnostic methods

Serological diagnosis of *B. burgdorferi* sl in horses can be carried out by a ELISA using the C6 antigen. Sensitivity and specificity, relatively to WB, are reported as 100% and 95%, respectively (Hansen et al., 2010).

Even in cases where the horses had no clinical signs, antibodies were detected within 5-6 weeks from infection. Antibody levels rose to a maximum over the following 3-4 months after which they remained static for at least 9 months. The antibody levels in horses were detectable for up to 2 years (Hansen et al., 2010).

A commercial rapid test, using the C6 antigen was shown to have 63% sensitivity and very high specificity (100%) for horses acutely infected with *B. burgdorferi* when compared to WB and a C6-ELISA test (Johnson et al., 2008).

Data submission

Number of tested and positive horses should be reported. Data relative to animals raised in herds should be accompanied by information on the number of tested herds as well as herds where at least one positive animal was found. Moreover, a distribution of herd size (number of animals per herd) should be reported.

1.1.8. Data collection on *B. burgdorferi* sl in domestic ruminants

Cattle, sheep, and goats develop antibodies against *B. burgdorferi* sl, and have the advantage of being easily accessible for data collection. Additionally, these animals are commonly sampled for monitoring of other diseases, and populations of these species are recorded by competent authorities. As a consequence, harmonised data collection is possible, but it should be limited to outdoor husbandry methods in areas where the climate is favourable to tick vectors. Commercial, diagnostic tests specifically developed for ruminants are not available, but home-made tests can be profitably used. The course of disease is chronic, subclinical and sometimes with no pathological signs. Only exceptionally is it possible to observe lameness, arthritis with pain and persistent low fever (Trávnicek et al., 2002). Only active data collection is proposed for domestic ruminants due to variable clinical signs caused by *B. burgdorferi* sl.

Specification on active data collection on B. burgdorferi sl in domestic ruminants.
Subpopulations to be covered

Cattle, sheep, and goats with frequent access to outdoor environment, below 1000 m asl should be considered, such as free-ranging herds.

Agents and possible subtypes

All genospecies belonging to *B. burgdorferi* sl should be considered.

Sampling context and design, sampling stage

Sampling may occur at the farm level. Multistage sampling can be implemented by randomly selecting herds from a list, and then testing individuals within each herd. As a consequence, clustering of observations needs to be taken into account when determining sample size, as described for horses (see Annex E for details on sampling). After herd selection sera can be tested for antibodies against *B. burgdorferi* sl (see below) among those that were collected for other disease control programs (i.e. brucellosis, Infectious Bovine Rhinotracheitis, etc.). Stratification at the first order administrative level, or, if possible, at the second order administrative level is recommended.

Type of specimen to be taken and sampling technique

Blood serum for subsequent antibody detection tests.
Diagnostic methods

Whole-cell *B. burgdorferi* antigens have been used in indirect fluorescent antibody (IFA) staining (Ortuño et al., 2003), immunoblotting, and ELISA methods in studies of cattle (Trávnicek et al., 2002), but there is little information available on the performance and suitability of ELISA with recombinant antigens for these animals. Humoral immune responses to *B. burgdorferi* sl in experimentally infected cattle appeared 14 to 51 days after inoculation (Tuomi et al., 1998). In another experimental study reactions in IFA tests, in dilutions 1 : 100, were observed from 17 to 21 days (Borko, 2008). Titres of antibodies in cattle in endemic areas persist as a result of frequent reinfections.

No commercial kits specific for these animal species is available. Tests using C6 peptide as an antigen might reasonably be adapted for use in ruminants, but no published report has been found.

Data submission

Number of tested and positive animals should be reported, separately for different animal species. Data relative to animals raised in herds should be accompanied by information on the number of tested herds and herds where at least one positive animal was found. Moreover, a distribution of herd size (number of animals per herd) should be reported. Sample size determination can be found in Annex E.

Table 5. Summary of proposals for active data collection on *B. burgdorferi* sl in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimes	Diagnosis
Dog	Animals frequenting outdoor habitat < 1000 m altitude	By private veterinarians based on owner compliance	Convenience sampling or random sampling. Stratified by administrative divisions. Multistage sampling in farm animals	Blood serum	Antibody detection test using C6 peptide as antigen[(a)]
Horse					
Domestic ruminants		At farm			

(a) Sensitivity and specificity of indirect diagnosis based on C6 antigen are not available for ruminants

1.2. References on *B. burgdorferi* sl

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2. FRANCISELLA

Francisella tularensis is a gram negative bacillus, facultative intracellular pathogen, which is able to infect a wide number of animal hosts. It causes tularemia, and it is considered one of the most infectious known pathogens (Ellis et al., 2002; Sjöstedt, 2007; WHO, 2007; Oyston, 2008). Four subspecies of *F. tularensis* are recognized (Sjöstedt, 2007): *F. tularensis* subspecies *mediasiatica*, *F. tularensis* subspecies *novicida* (or *F. novocida*, as a separate species), *F. tularensis* subspecies *tularensis*, *F. tularensis* subspecies *holarctica* (Johansson et al. 2010, Huber et al. 2010, Busse et al., 2010).

Only *F. tularensis* subspecies *tularensis*, and *F. tularensis* subspecies *holarctica* are of zoonotic importance and are commonly referred to as *F. tularensis* type A or type B, respectively. The two subspecies do not show antigenic differences. However, they greatly diverge on geographic distribution and virulence. *F. tularensis* type A, which is present in North America, is characterised by a moderate to extreme virulence for humans and domestic rabbits, and can cause disease in several species of domestic animals. *F. tularensis* type B, which is present in Europe, Asia, and, in a lesser extent, in North America, is less virulent for humans and domestic rabbits, and it can cause severe clinical signs and mortality in wild rodents and hares (Jellison, 1974; Sjöstedt, 2005). The most common forms of human tularemia are the ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, and the pneumonic forms.

2.1. Inventory of available data on *Francisella* in animals

2.1.1. Transmission cycle of *F. tularensis*

The number of animal hosts for *F. tularensis* is exceptionally high. One hundred forty five vertebrates and 111 invertebrate species are currently known to be susceptible to the infection (Oyston, 2008). Ticks, flies, and mosquitoes are the most important invertebrate vectors (Petersen et al., 2009). The transmission cycle and maintenance of *F. tularensis* in nature is not completely clarified (Tärnvik et al., 2004). In fact, in addition to the role of rodents and lagomorphs as reservoir hosts, recent evidence suggests an additional phase of the life cycle of *F. tularensis* in aquatic environments. Fresh water protozoa, in particular amoebae, have been proposed to act as reservoir of *F. tularensis* (Abd et al., 2003; Schmitz-Esser et al., 2010, Margolis et al, 2010). Under these circumstances, rodents and lagomorphs should be more appropriately defined as amplifying hosts.

Animals and man can be infected either directly, though close contact with other, infected animals or their contaminated tissues or fluids, or indirectly, through inhalation of infective aerosols, ingestion of contaminated water or food and through ticks or insect's bites (Hopla, 1974; Hubálek et al., 1996; Willke et al., 2009; Hauri et al., 2010; Moniuszko et al., 2010).

Vectors become infected directly through blood feeding from infected animals or, in the case of mosquitoes, from contaminated water (Friend, 2006). Transtadial but not transovarial transmission has been demonstrated among vectors (Hopla, 1960; Hubálek et al., 1996, Petersen et al, 2009). Human to human transmission has not been documented (WHO, 2007).

Reservoir/amplifying hosts include in particular lagomorphs, rodents and, to a minor extent, insectivores (Henson et al., 1978; Mörner et al., 1988; Gurycová et al., 2001; Petersen et al., 2004; Friend, 2006). Much less common hosts are birds, reptiles, amphibians, crustaceans, mollusks, fish and annelids (McKeever et al., 1958; WHO, 2007; Padeshki et al., 2010).

Unlike reservoir hosts for other agents (such as *B. burgdorferi* sl and WNV) reservoir/amplyfing hosts for *F. tularensis* usually develop acute infections that may cause severe clinical and pathological signs and death, possibly in outbreak patterns. Lagomorphs and rodents normally die 3-8 days post-infection. After infection, high levels of bacteria develop in their internal organs, tissues and blood. Such high levels ensure infection of the arthropod vectors that feed on them. In fact, these animals can

be heavily parasitized by *Dermacentor* spp., *Haemaphysalis* spp., and *Ixodes* spp. ticks (Hopla, 1960; Gurycová et al., 2001). The bacteremia also promotes the contamination of terrestrial and aquatic environments via feces, urine or carcasses (Friend, 2006).

Low antibody titres against *F. tularensis* have been found in clinically healthy European brown hares (*Lepus europaeus*) and voles (*Microtus rossiaemeridionalis*, *M. arvalis*) from Eastern European regions (Bell and Stewart, 1975; Olsufjev et al., 1984; Treml et al., 2007). Furthermore, hares are able to excrete live *F. tularensis* with urine during interepizootic periods (Gyuranecz et al., 2010a). These findings suggest a potential role of chronic forms of infection in reservoir/amplifying hosts in the persistence of *F. tularensis* in endemic areas.

In a recent study in Hungary, Gyuranecz et al. (2011) concluded that, during inter-epizootic periods, *F. tularensis* persists in a transmission cycle including the European brown hare and the hard tick *Haemaphysalis concinna* (that may harbor the bacteria for several years, across multiple life stages); conversely, at the same location, small rodents are not considered as true reservoir hosts.

Dead end hosts include wild and domestic ruminants, swine, and carnivores. Although these species do not develop clinical signs after infection by *F. tularensis*, they develop detectable antibody response. It must be mentioned that *F. tularensis* DNA has been sporadically detected, by PCR, in roe deer, rabbits, and wild boars which were found clinically ill or dead in France[9]. Although causal association between the agent and disease could not be demonstrated in these cases, further investigations are needed.

Indirect transmission through bites of infected ticks and insects, from contaminated pastures, water, or infected rodents are the most frequent infection routes for dead end hosts (Zidon, 1964). Wild carnivores and scavenger species (i.e. foxes, coyotes, raccoons) can become infected from eating moribund or dead animals (Al Dahouk et al., 2005; WHO, 2007; Olsen 1975; Bell, 1980; Bischof and Rogers, 2005). Immunity following infection of the wild carnivores is frequently lifelong (Friend, 2006).

Birds are fairly resistant, but a variety of species have acquired natural infection and mortality has been reported in some instances (Hopla and Hopla, 1994). Padeshki et al. (2010) reported the case of a hunter that developed tularemia following the scratch from a hawk (*Buteo buteo*), that is a bird species often preying on rodents and lagomorphs.

The transmission cycle of *F. tularensis*, with emphasis on the role of animals, is represented in a diagram in Figure 3. Transmission of *F. tularensis* (represented by arrows) between reservoir/amplifying hosts and other environmental sources (water and arthropod vectors) ensures maintenance of the agent.

[9] SAGIR. National French Network of disease surveillance in wildlife. ONCFS/FNC/FDC.

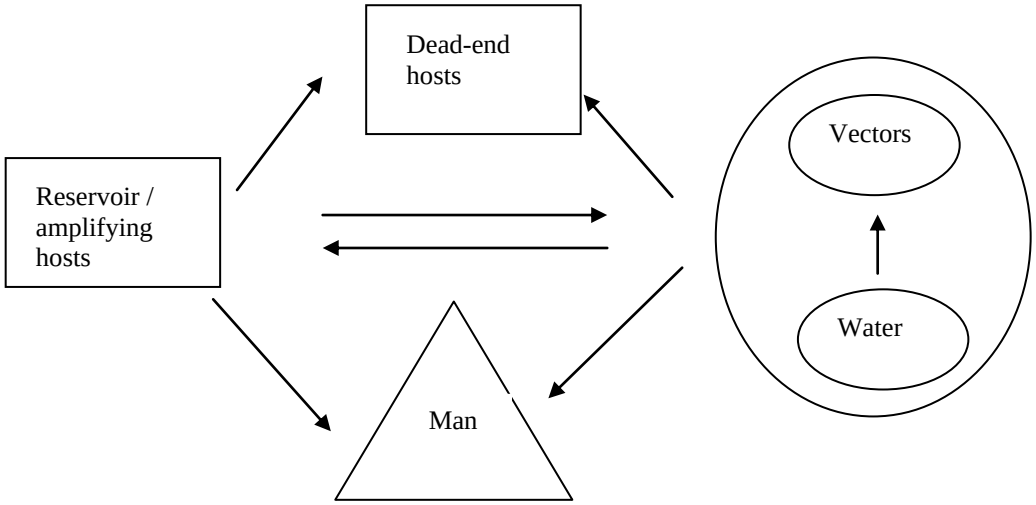

Figure 3. Diagram of the transmission of *F. tularensis* with specific focus on the role of animals. Specific information on host and vector species can be found in the text.

2.1.2. Diagnosis of *F. tularensis* in animals.

Clinical signs of F. tularensis in animals.

In wild animals clinical signs of tularemia are rarely observed. In fact, reservoir/amplifying hosts are frequently found dead or moribund (Friend, 2006). In hares, clinical signs can range from exhaustion, tameness, stupor, depressed behavior, rubbing nose and forefeet into the ground, recurrent spasm, staggering (Bell, 1980; Bell and Reilly, 1981; Mörner and Addison, 2001). No clinical sign caused by *F. tularensis* type B is usually reported in other animal species, such as ruminants, pigs, dogs.

Laboratory diagnosis of F. tularensis in animals

Direct laboratory diagnosis

Direct methods used to detect *F. tularensis* in animals include culture and mice inoculation, PCR, capture enzyme linked immunosorbent assay (cELISA), immunofluorescence assay (IFA), and immunohistochemistry (IHC).

F. tularensis will not normally grow on ordinary media and it is necessary to use special culture media, such as Francis medium, McCoy and Chapin medium, Modified Thayer-Martin agar, GCA agar with tiamine (OIE, 2008). Culture allows the characterisation of bacteria, but sensitivity is low and it poses a high risk of laboratory infection (Splettstoesser et al., 2010).

Inoculation of mice with material from pathological specimens can be used for the direct detection of *F. tularensis*. All routes of administration in mice, such as subcutaneous, percutaneous, or intravenous, will lead to an infection that is fatal within 2–10 days (OIE, 2008). This technique is extremely hazardous and it is only recommended for agent identification in cases when culture is negative and agent identification is needed for epidemiological reasons. It should only be undertaken where proper biosafety facilities (level 3) are available.

PCR has several advantages over the other methods for the detection of *F. tularensis*, such as bacterial culture and mice inoculation, since difficulties in the isolation of this fastidious agent and risk for laboratory personnel are avoided. Targets for amplification include genes encoding for outer membrane proteins such as *fop*A, and *tul*4, and for a 23 kDa protein which is associated with infection of macrophages. The amplification and sequencing of the 16S rRNA gene can also be useful. Greater sensitivity and specificity are obtained by using the *tul*4 gene (Sjöstedt et al., 1992; Long et al., 1993).

Real-time PCR methods are particularly rapid and sensitive. A highly sensitive and specific multi-target real-time TaqMan PCR, targeting genes for ISFtu2, 23 kDa, tul4, fopA, was used on animal samples (Versage et al., 2003). A very useful tool for epidemiological investigations are the Multi-Locus-Variable number tandem repeat Analysis (MLVA) which allowed discriminating among different strains (Farlow et al., 2001; Johansson et al., 2004).

Direct immunofluorescence test is carried out on tissues fixed in formalin and paraffin. It is a rapid technique but it does not allow strain characterization. (OIE, 2008; Petersen et al., 2004).

Capture enzyme linked immunosorbent assay (cELISA) is used in antigen detection and is characterised by good sensitivity and specificity (Grunow et al. 2000). It can be used on animal tissues and also on environmental samples such as water and mud (Berdal et al., 2000; Grunow et al., 2000).

Immunohistochemistry (IHC) can be applied for the demonstration of *F. tularensis* lipopolysaccharide antigen in tissue sections. Several authors have shown the IHC assay to be a useful and sensitive method for the detection of *F. tularensis* in domestic and wild animals (Gyuranecz et al., 2010a; Twenhafel et al., 2009; Zeidner et al., 2004).

Indirect laboratory diagnosis

Animal species which suffer clinical signs from the infection, such as amplifying hosts (rodents, lagomorphs) often die before specific antibodies develop. Conversely, significant titers can be found in more resistant dead end hosts, such as ruminants, swine, dogs, and birds, and also in chronically infected hares.

Indirect methods used to detect *F. tularensis* in animals include tests for IgG antibody detection in blood, plasma or serum samples: tube agglutination or microagglutination test (MAT), enzyme linked immunosorbent assay (ELISA) and immunochromatographic assay.

Tube agglutination and microagglutination tests (MAT, in microtitration plates) are commonly used serological tests, accurate, rapid, and economic (Brown et al., 1980; Koskela end Herva, 1982; Sato et al., 1990). A culture of *F. tularensis* on specific medium is used as antigen. A titer 1:40 is to be considered doubtful while 1:80 should be considered as positive. Agglutination tests mainly detect IgM immunoglobulins that are richer in agglutinins in comparison with IgG and IgA (Koskela and Salminen, 1985). Although 100% sensitivity and specificity have been obtained by using MAT in humans (Porsch-Ozcürümez et al., 2004), cross reaction with *Brucella* spp and *Yersinia* spp. may occur.

Enzyme linked immunosorbent assay (ELISA) is used in early diagnosis of tularemia and identifies IgG, IgM and IgA (OIE, 2008). It shows high sensitivity and specificity (Syrjala etal., 1985; Splettstoesser et al., 2010).

An immunochromatographic test to detect IgG antibodies specific to *F. tularensis* in serum from humans and other mammal species (primates, pigs, and rabbits) was recently developed. This new tool requires minimal laboratory equipment, and the results are obtained within 15 min. When applied to animal sera, and by using MAT as the gold standard, it was 100% sensitive and specific (Splettstoesser et al et al., 2010).

2.1.3. Data collection on *F. tularensis* in animals by competent authorities of EU countries, Norway and Switzerland.

Summary of results of questionnaires to the Task Force on zoonoses data collection

Fourteen countries responded to the questionnaires regarding *F. tularensis*: Denmark, Estonia, Finland, France, Hungary, Italy, Latvia, Portugal, Romania, Slovakia, Slovenia, Sweden, Norway and Switzerland.

Tularemia is a notifiable disease in animals in nine of the responding countries: Denmark, Estonia, Finland, France, Italy, Latvia, Slovenia, Norway and Switzerland, whereas it is not notifiable in Hungary, Portugal, Romania and Slovakia.

Active data collection in animals is carried out in Hungary, Italy, and Sweden.

Passive data collection in animals is carried out in Denmark, Finland, France, Italy, Latvia, Norway, Slovakia, Slovenia, Sweden, and Switzerland.

In Estonia, Romania and Portugal no data collection activity (active/passive) in animals is carried out.

Country by country results

Country by country results are reported in Annex A. Passive and active data collection activities on *F. tularensis* are summarised in Table 6

Table 6. Summary of results of the questionnaire on active and passive data collection on *F. tularensis* in animals[(a)]

Country	Active data collection			Passive surveillance			National Reference Laboratory
	Time span	*Animal species*	*Diagnosis*	*Time span*	*Animal species*	*Diagnosis*	
Denmark							National Veterinary Institute Technical University of Denmark
Estonia							Estonian Veterinary and Food Board
Finland				1980 – present	Hares	Antigen-detection IFA	Finnish Food Safety Authority
France				1986 – present	Hares	PCR	Maisons-Alfort laboratory for studies and research on animal disease and zoonoses
Hungary	1980 – present	Hares, other species	MAT, IHC				Central Agricultural Office Veterinary Diagnostic Directorate
Italy	2004 – present	Hares	PCR, MAT, bacterial isolation	1990 – present	Hares, wild boars, sheep, foxes, deer and others	PCR, MAT, bacterial isolation	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
Latvia							Food and Veterinary service
Norway					Hares	PCR	Norwegian Food Safety Authority
Portugal							Laboratório Nacional de Investigação Veterinária
Slovakia				1987 – present	Hares, rabbits, deer	MAT	
Slovenia					Hares	PCR	Veterinary Administration of Republic of Slovenia
Sweden	2003-present	Rodents, insectivores	PCR	1980-present	Hares	Antigen-detection IFA	National Veterinary Institute
Switzerland					All susceptible species	PCR, bacterial isolation	University of Bern, Vetsuisse Faculty

(a) No data collection activities nor reference laboratory were reported in the questionnaire from Romania. MAT: microscopic agglutination test, IHC: immunohystochemistry.

2.1.4. Systematic literature review of data collection on *F. tularensis* in animals

The literature search was carried out on publications from 1990 through 2010. Results by country, information on diagnostic tests and sampling, as well as specific bibliographic references are presented in Annex B.

Results of active data collection on F. tularensis by animal species

Main results of active data collection are summarised in Table 7. Thirty four publications were retrieved on active data collection on animals. Of these publications, only twenty six included data on the number of tested and of positive animals and, therefore, were used to estimate prevalence. Publications on active data collection were from 12 countries: Austria, Bulgaria, Czech Republic, France, Germany, Hungary, Italy, Norway, Slovakia, Spain, Sweden, and Switzerland.

Small mammals were the object of data collection in the greater number of countries; prevalence of *F. tularensis* was generally low, even in studies carried out in areas where the infection is considered as endemic. This could be explained by the high fatality of *F. tularensis* in these animal species (Kaysser et al., 2008). It is, however, to be noted that *F. tularensis* DNA was found in 23.5% of 136 black rats (*Rattus rattus*, grouped among small mammals in Table 7) that were tested in Bulgaria by Christova and Gladnishka (2005). Relatively greater prevalence than in small mammals was found in hares, suggesting that chronic infections might be more frequent in this species.

Among domestic animals, relatively high prevalence was found in horses and in goats (27.6% and 26.7% respectively) in studies that were carried out in Italian locations after the report of human cases of tularemia.

Dogs were found positive in different countries (overall prevalence = 14.1%), whereas lower prevalence levels were reported on other species such as sheep and cattle.

Among wild dead end hosts, prevalence reported from wild boars and foxes is lower than prevalence in domestic species, in spite of an allegedly greater probability of contact with wild reservoir hosts. Such variation in prevalence estimates might be explained by different levels of transmission of *F. tularensis* across study locations, or by variations in the duration of antibody response across animal species (this information is, however, not available).

Serological examination of Cynomolgus Monkeys (*Macaca fascicularis*), from a population that was struck by an outbreak of *F. tularensis* in a zoo in Germany, was included in active data collection and, therefore, results are presented in Table 7 (Primates). The high prevalence level (51.4%) must be interpreted by taking into account these very specific circumstances.

Prevalence estimates (and associated confidence intervals) that were obtained from the systematic literature review provided information on real scenarios, suggesting that the continuous data collection on animals can be useful to analyse the increasing trend of *F. tularensis* in European countries (Kaysser et al., 2008), and to detect the agent's circulation in the environment even before the occurrence of outbreaks in humans. On the other hand, the reviewed data collection activites were carried out with variable objectives, by using different diagnostic tests, and, in certain cases, targeted to specific animal populations considered at high risk of infection. As a consequence, results cannot be generalised at the level of countries or groups of countries.

Table 7. Results of active data collection on *F. tularensis* in animal species based on the systematic review of publications in the EU, Norway and Switzerland, from 1990 through 2010[(a)].

Species	N of countries	N of studies	N tested animals	N of positive animals	Prevalence	Lower 95% CL	Upper 95% CL
Small mammals[(b)]	8	77	4998	111	2.22	1.02	4.77
Hare[(c)]	6	14	1285	70	5.45	2.19	12.89
Wild boar[(d)]	3	9	1118	49	4.38	2.37	7.96
Mustelid[(e)]	3	5	8	1	12.50	1.15	63.61
Dog[(f)]	3	3	644	91	14.13	8.60	22.34
Bird[(g)]	2	5	22	0	0.00	0.00	0.15
Sheep[(h)]	2	3	1237	24	1.94	1.28	2.93
Badger[(i)]	2	3	4	0	0.00	0.00	0.60
Deer[(j)]	1	3	61	3	4.92	3.39	7.09
Fox[(k)]	1	3	16	0	0.00	0.00	0.21
Cattle[(l)]	1	2	2835	86	3.03	0.35	22.01
Rabbit[(m)]	1	2	262	0	0.00	0.00	0.01
Pig[(n)]	1	2	240	2	0.83	0.12	5.72
Goat[(o)]	1	2	30	8	26.67	12.98	46.99
Petting zoo animals[(p)]	1	1	423	0	0.00	0.00	0.01
Eurasian lynx[(q)]	1	1	91	0	0.00	0.00	0.04
Horse[(r)]	1	1	76	21	27.63	18.76	38.70
Primates[(s)]	1	1	35	18	51.43	35.30	67.26
Shellfish[(t)]	1	1	20	0	0.00	0.00	0.17
Buffalo[(u)]	1	1	2	0	0.00	0.00	0.84
Fish[(v)]	1	1	2	0	0.00	0.00	0.84
Muflon[(w)]	1	1	2	0	0.00	0.00	0.84
Cat[(y)]	1	1	1	0	0.00	0.00	0.98

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study. (b) Anda et al., 2001 , Berdal et al., 1996, Berdal et al., 2000, Christova and Gladnishka, 2005, Gurycová et al., 2001, Kaysser et al., 2008, Magnino et al., 1990, Petersen et al., 2004, Splettstoesser et al., 2007, Treml et al., 2001, Vidal et al., 2009; (c) Anda et al., 2001, Dedek et al., 1990, Ercolini et al., 1991, Frölich et al., 2003, Gyuranecz et al., 2010b: in this study, seropositive hare were tested by culture and PCR and, therefore, prevalence might be overestimated, Haerer et al., 2001, Hauri et al., 2010, Hubálek et al., 1993, Magnino et al., 1990, Zanni et al., 1995 ; (d) Al Dahouk et al., 2005, Ercolini et al., 1991, Hubálek et al., 1993, Hubálek et al., 2002, Magnino et al., 1990 ; (e) Berdal et al., 1996, Gurycová et al., 2001, Magnino et al., 1990; (f) Gurycová et al., 1992, Magnino et al., 1990; (g) Magnino et al., 1990, Siret et al., 2006 ; (h) Anda et al., 2001, Ercolini et al., 1991, Magnino et al., 1990; (i) Anda et al., 2001, Magnino et al., 1990; (j) Hubálek et al., 1993; (k) Hubálek et al., 1993; (l) Ercolini et al., 1991, Magnino et al., 1990; (m) Ercolini et al., 1991, Magnino et al., 1990; (n) Ercolini et al., 1991, Magnino et al., 1990; (o) Ercolini et al., 1991, Magnino et al., 1990; (p) Butikofer et al., 2005; (q) Ryser-Degiorgis et al., 2005; (r) Ercolini et al., 1991; (s) Mätz-Rensing et al., 2007; (t) Anda et al., 2001; (u) Magnino et al., 1990; (v) Anda et al., 2001; (w) Hubálek et al., 1993; (y) Magnino et al., 1990.

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Results of passive data collection on F. tularensis by animal species

Passive data collection on *Francisella tularensis* in animals is summarised in Table 8. Publications are from five countries.

Table 8. Results of passive data collection on *F. tularensis* in animal species based on the systematic review of publications in the EU, Norway and Switzerland, from 1990 through 2010.

Species	Country	N of studies	N of suspected cases	N of confirmed cases
Apodemus spp.[(a)]	Italy	1	26	0
Callithrix jacchus[(b)]	Germany	1	7	5
Callithrix jacchus[(c)]	Switzerland	1	1	1
Chlorocebus aethiops and *Erythrocebus patas*[(d)]	Hungary	1	NA	2
European hedgehog[(e)]	Italy	1	1	0
Game birds[(f)]	Italy	3	23	0
Hare[(g)]	France	18	NA	117
Hare[(h)]	Germany	1	1	1
Hare[(i)]	Italy	1	8	0
Macaca fascicularis[(j)]	Germany	1	6	6
Red squirrel[(k)]	Italy	1	2	0
Wild boar[(l)]	Italy	1	6	0
Turdus merula[(m)]	Italy	1	2	0

(a) Ercolini et al., 1991; (b) Splettstoesser et al., 2007 ; (c) Posthaus et al., 1998; (d) Gyuranecz et al., 2009; (e) Ercolini et al., 1991; (f) Ercolini et al., 1991; (g) Vaissaire et al., 2005, Mailles et al., 2010; (h) Muller et al., 2007; (i) Ercolini et al., 1991; (j) Mätz-Rensing et al., 2007; (k) Ercolini et al., 1991; (l) Ercolini et al., 1991; (m) Ercolini et al., 1991; NA: not available

2.1.5. Information on the occurrence and geographical distribution of *F. tularensis*, host and vectors

Agent

F. tularensis is widespread across Europe, and it has been reported in many countries of the northern hemisphere. The infection was never reported in the United Kingdom and Ireland (WHO, 2007).

Geographic distribution of results of *F. tularensis* in animals from the systematic literature review is summarised in Figure 4. Countries where positive animals were reported, either by active or passive data collection, are classified as positive. Sweden is classified as negative since all tested animals (91 Eurasian lynx) resulted as negative. Details by country and animal species can be found in Annex B.

In certain countries, *F. tularensis* was reported in publications that were not included in this systematic literature review. As an example, infection was found in Sweden (in hares) by Mörner et al. (1988), but this publication was outside the time range of our systematic literature review. Moreover, the infection was detected in Finland (in hares and squirrels), according to the "Reports of the meeting of the OIE working group on wildlife diseases", from 2003 to 2008 (http://www.oie.int/?id=438); lack of information prevented the use of these data in the systematic literature review.

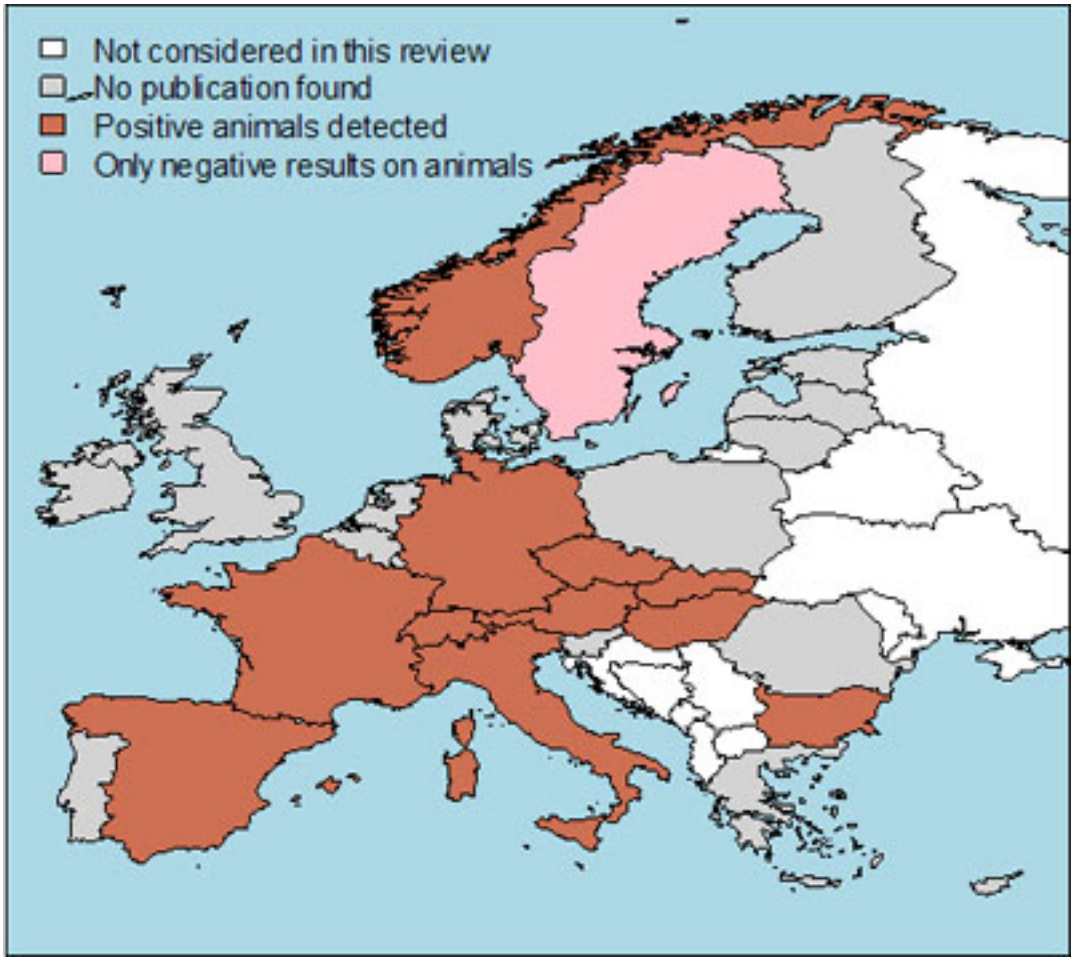

Figure 4. Geographic distribution of *F. tularensis*, based on active and passive data collection on animals, from the systematic literature review of publications from 1990 through 2010[10].

Hosts

See general section on distribution of hosts for vector borne agents in Annex F.

Vectors

The geographical distribution of *I. ricinus* is described in the section on *Borrelia*.

The geographic distribution of *Dermacentor marginatus* is published in the scientific opinion EFSA (EFSA 2010a). The geographic distribution of *Haemaphysalis concinna* is published in the scientific opinion EFSA (2010b). The geographic distribution of mosquitoes is described in the section on WNV.

2.2. Proposal for data collection on *F. tularensis* in animals

The application of criteria for the selection of animal species to be proposed for data collection can be summarised as follows (the application of criteria can be seen in detail in Annex C).

[10] Austria: Gurycová et al., 2001; Bulgaria: Christova and Gladnishka, 2005; Czech Republic: Hubálek et al., 1993, Hubálek et al., 2002, Petersen et al., 2004, Treml et al., 200; France: Siret et al., 2006, Vaissaire et al., 2005; Germany: Al Dahouk et al., 2005, Dedek et al., 1990, Hauri et al., 2010, Kaysser et al., 2008, Mätz-Rensing et al., 2007, Muller et al., 2007, Splettstoesser et al., 2007; Hungary: Gyuranecz et al., 2009, Gyuranecz et al., 2010b; Italy: Ercolini et al., 1991, Magnino et al., 1990; Norway: Berdal et al., 1996, Berdal et al., 2000; Slovakia: Gurycová et al., 1992, Gurycová et al., 2001; Spain: Vidal et al., 2009; Swede: Ryser-Degiorgis et al., 2005; Switzerland: Haerer et al., 2001.

Since reservoir/amplifying hosts develop clinical disease and may rapidly die following infection by *F. tularensis*, these species are suitable for passive data collection. Conversely, active data collection can be hampered by the reduced probability of sampling (for example, by trapping) moribund or dead animals. This is supported by results of the systematic literature review, showing a generally low prevalence of *F. tularensis* in small mammals by active data collection (even when certain endemic areas were considered. As a consequence, and considering also the need to capture these animals and test them by direct laboratory methods, that might detect the infection before the development of clinical signs (Kaysser et al., 2008), active data collection on *F. tularensis* in small mammals is not proposed. An exception could be made for small mammals that are captured for data collection on other agents, such as Hantavirus, Leptospira, and TBEV. In these cases, additional testing for *F. tularensis* can be considered (see specific sections on those agents for details).

Hares may be more resistant than other species (such as small rodents) and infection may have a longer duration. Furthermore, hares are hunted and are the object of management and farming in certain areas, and are moved among European countries for repopulation and hunting purposes. Under these circumstances, active data collection can be proposed on hares at the export stage and during hunting (see below for details).

Dead end hosts have several advantages as target for active data collection, including widespread distribution in Europe and development of long-lasting antibody response in the absence of clinical signs. By means of the continuous data collection on dead end hosts, circulation of *F. tularensis* could be detected before human cases are detected. Based upon the systematic literature review, several publications report on the detection of antibodies against *F. tularensis* in dead-end hosts (Table 7).

The possibility of harmonised, active data collection would be favoured on domestic dead-end hosts, such as cattle, sheep, goats, and pigs among farm animals, and dogs and horses among companion animals. Nevertheless, for those areas where wild dead-end hosts, such as wild boars and foxes, are regularly hunted, data collection on these species can be advantageous, especially in terms of sensitivity in the detection of the circulation of *F. tularensis* in the environment.

The planning of data collection from dead-end hosts, and the interpretation of results would greatly benefit from research aimed to the possible transmission routes of *F. tularensis* to dead-end hosts. The effects of variable parameters associated with these transmission routes are analysed in the dynamic model described in Annex D.

Passive data collection from dead-end hosts is not proposed due to the absence of clinical signs attributable to *F. tularensis* type B (circulating in Europe).

Proposals for data collection on *F. tularensis* in animals are summarised in Table 9 and Table 10.

2.2.1. Data collection on *F. tularensis* in hare and wild rodents.

Specifications on active data collection on F. tularensis in hares

Subpopulations to be covered

Active data collection on hares should be targeted toward hares that are moved from different geographic areas or countries for repopulation or hunting purposes, and those that are killed during hunting. In fact, export and hunting are situations in which hares are normally handled and the collection of specimens is relatively feasible.

Agents and possible subtypes

F. tularensis type B is present in Europe. Diagnostic tests are, however, able to detect infection by all subspecies.

Sampling context and design, sampling stage

Hares sould be sampled at the time of export from an area, and during hunting.

The objective of sampling would be the detection of markers of *F. tularensis* infection in hares. Sample size should, therefore, be based on the minimum prevalence level of the infection to be detected. Based on results of the systematic literature review, where prevalence was estimated as 5.5%, testing 70 animals (assuming a large population in the area of origin) would correspond to 95% confidence of detecting at least one infected animal. It is to be noted that this calculation assumes perfect sensitivity and specificity of laboratory tests. Furthermore, selecting animals from those that are exported and from those that are killed during hunting (constituting the available study population) cannot be considered as a random sample of the target population of an area. Consequently, conclusions on the presence of *F. tularensis* must be drawn with caution. The same considerations apply when calculating country-level prevalence from these data collection activities, and non independence of observations from hares in the same geographic areas should be taken into account.

Type of specimen to be taken and sampling technique

Blood for serological tests for the detection of antibodies against *F. tularensis* could be collected from both live and dead hares. Blood collection from live hare should be carried out by veterinarians or trained technicians.

Diagnostic methods to be used

Indirect laboratory tests should be used in active data collection in hare, where MAT is recommended.

Data submission

Number of exported lots of hares, and number of tested hares, should be reported to account for clustering in statistical analysis.

Specifications on passive data collection on F. tularensis in hares and wild rodents

Subpopulations to be covered

No distinction among subpopulations is made for passive data collection, which should consider all reservoir/amplifying hosts. Animal species to be considered include lagomorphs, wild rodents, sciurids, and insectivores.

Sampling context and design, sampling stage

Suspect cases should be reported by veterinarians and, in general, personnel involved in wildlife management and monitoring (including wildlife protection institutions, hunting associations). As specified in the definition section, reporting of dead or clinically ill wild animals, as carried out by personnel engaged in other activities (that is, not specifically going outdoors to look for dead or ill animals) is classified as part of passive data collection in this report. The above mentioned personnel should inform health authorities (i.e. veterinarians, reference laboratories) of the finding. Owing to risks of infection by handling hares and rodents infected with *F. tularensis*, the collection of dead or ill animals should be solely carried out by authorised personnel, such as veterinarians or technicians.

All suspect cases should be considered in passive data collection, based on clinical signs and pathological lesions in amplifying/reservoir hosts, including small rodents and hares. Typical pathological lesions visible on the surface of the liver, spleen, and lymph nodes are slightly raised necrotic foci and the spleen and liver may have a dark bluish-red coloration and be enlarged (Reilly, 1970; Omland et al., 1977). Granulomatous lesions can be found on kidneys, lungs, liver, and in subcutaneous areas, especially in chronic infections in geographic areas where *F. tularensis* is endemic (Gyuranecz et al., 2010a; Bell and Stewart, 1975). In hares, it is always important to carefully examine the animal to search for the presence of ticks, and always evaluate the most common differential diagnoses such as EBHS (European Brown Hare Syndrome), brucellosis, pseudotuberculosis, toxoplasmosis, pasteurellosis and abscess due to staphylococcosis.

Type of specimen to be taken and sampling technique

Whole animals are to be collected. Spleen, liver, lung, kidney are organs to be analysed in laboratory.

Diagnostic methods to be used
PCR and bacterial culture, and subsequent characterization are recommended.

2.2.2. Data collection on *F. tularensis* in dead end hosts.

Specifications on active data collection on F. tularensis in dead-end hosts
Dead-end hosts to be considered include the following species: sheep, goats, cattle, horses, pigs, and dogs among domestic animals, wild boars and foxes among wild animals.

Subpopulations to be covered

Free ranging farm animals, i.e.: those grazing outdoors for at least part of the year and companion animals frequenting outdoor habitat should be included in active data collection. Continous (i.e. yearly) sampling of animals of varying age would provide useful information. Specifically, testing young indiduals would favor the detection of the recent circulation of *F. tularensis* in the environment (as a possible consequence of outbreaks in reservoir/amplyfing hosts), whereas past exposure to the infection would only be detected in older individuals.

Sampling context and design, sampling stage

Sampling can be performed at farm or by veterinarians with owner compliance for companion animals, and during hunting (or trapping) for wild animals. If animals are sampled within the context of data collection for other diseases, blood sera from the same animals can be used for testing for antibodies against *F. tularensis*. Stratification at the level of the second order administrative division would improve representativity of sampling (see Annex E).

Since the objective of sampling would be the detection of *F. tularensis* infection circulating in dead-end hosts, sample size will be determined based upon minimum prevalence level to be detected as an indicator of the infection in the environment (reservoir/amplifying hosts, vectors, water). In systematic literature review, prevalence as low as 4.4% was found in wild boar, and detection of infection at such low levels would require ~ 85 animals. On the other hand, in order to detect a prevalence of 10% or greater (as observed in several dead end host species), 30 animals should be tested to have 95% confidence of detecting at least one infected animal. The same assumptions and considerations mentioned in the proposal for active data collection in hare apply also to these sample size determinations.

Type of specimen to be taken and sampling technique

Blood serum is to be collected and stored for analysis.

Diagnostic methods to be used

Only indirect laboratory diagnosis is proposed, by using MAT

Data submission

For farm animals, number of farms, and number of tested animals within farms should be reported to account for clustering in statistical analysis. In areas where *F. tularensis*-positive dead end hosts were previously found, reporting of age of tested animals would favor the detection of recent cases of infection in young individuals, as the likely consequence of recent circulation of the agent in the environment.

Table 9. Summary of proposals for active data collection on *F. tularensis* in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimes	Diagnosis
Hare	Animals subject to movements across geographic areas, and hunted animals	Import or movement between countries or geographic areas, and during hunting	Stratified by administrative area. Convenience or random sampling based on available list. Multistage sampling for farm animals.	Blood serum	MAT
Wild boar, fox	Hunted or trapped animals	Hunting			
Dog	Frequenting outdoor habitat	Owner			
Farm animals: sheep, goats, cattle, horses, pigs	Free ranging	Farm			

Table 10. Summary of proposals for passive data collection on *F. tularensis* in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Type of specimens	Diagnosis
Wild lagomorphs, small rodents, sciurids, insectivores	Death, clinical signs and pathological lesions	Suspect cases reported by veterinarians, wildlife management personnel	Spleen, liver, lung, kidney	Bacterial culture, PCR

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3. LEPTOSPIRA

Leptospirosis is a zoonotic disease which is caused by spirochetes of the genus *Leptospira* and is considered as the most widespread zoonosis worldwide. In fact, almost all mammals and some reptiles are susceptible to the infection. Leptospirosis is re-emerging in both developed and developing countries (Waitkins, 1987; Faine, 1994; WHO, 1999; Levett, 2001; Meites et al., 2004; Pappas et al., 2008).

Taxonomy of *Leptospira* is complex and undergoing changes. On the other hand, it is important for ecological and epidemiological differences within the genus. The traditional serological classification differentiated two species: *Leptospira interrogans* and *Leptospira biflexa*. The former covers all pathogenic strains while the latter includes all saprophytic (i.e. growing on dead or decaying organic matter) strains (Faine and Stallman, 1982). *L. interrogans* and *L. biflexa* have, respectively, 250 and 45 serovars recorded. Closely related serovars are assembled into serogroups in such a way that all the 250 serovars of *L. interrogans* are arranged in 25 serogroups and all serovars of *L. biflexa* in 38 serogroups. However, serogroups do not have official taxonomic status and are mainly intended for laboratory use (Vijayachari et al., 2008). Recently, a genotypic classification of the genus has emerged claiming to be taxonomically more correct, as it is based on genetic homology (Levett, 2001). The reclassification of *Leptospira* in genotypic groups (named genomospecies) has brought some confusion. This is because there is no correspondence between genomospecies and the two traditional species. Indeed, both pathogenic and non-pathogenic serovars occur within the same genomospecies (Levett, 2001). Fifteen different genomospecies have been isolated from almost all warm-blooded animal species (Vijayachari et al., 2008). At present, both classification systems are being used. For this reason in this report we use the general term *Leptospira* for all leptospires infecting animals and we still use the serological classification for indicating specific serovar/serogroup of *Leptospira.*

3.1. Inventory of available data on *Leptospira* in animals

3.1.1. Transmission cycle of *Leptospira*

Transmission of *Leptospira* in animals and man occurs through direct or indirect contact with urine from infected animals (Vijayachari et al., 2008; Levett, 2001; Faine 1994). Cuts or abrasions in the skin, or the conjunctiva are the main entrance point for the bacteria (Levett, 2001). Water-borne transmission is reported (Jorge, 1932; Cacciapuoti et al., 1987), whereas transmission via animal bite is rare (Barkin et al., 1974; Luzzi et al., 1987; Gollop et al., 1993). In animals, direct transmissions can be transplacental, haematogenous, by milk from the infected mother, or by sexual contact (Ellis et al., 1985; Vijayachari et al., 2008). Direct transmission between animals and humans is linked with occupational exposure for farmers, veterinarians, slaughtermen, rodent control workers, butchers (Vijayachari et al., 2008). The transmission of *Leptospira* from adopted feral rats to humans is reported (Strugnell et al., 2009). Indirect transmission occurs by urine from infected animals contaminating rivers, water streams, ground water, soil and mud (Faine, 1994; Vijayachari et al., 2008).

There is a wide variety of animal species that serve as hosts for *Leptospira* (WHO, 2003; Vijayachari et al., 2008). When an animal is infected with *Leptospira*, the bacteria reach its kidneys and are shed in the urine (leptospiruria) in a continuously or intermittent manner (Babudieri, 1958). Rodents are the most widely distributed hosts of *Leptospira*, and they act as reservoirs for humans and domestic animals. Other animals, including insectivores (i.e. shrews and hedgehogs), domestic animals (i.e. cattle, pigs, dogs and to a lesser extent, sheep, goats, horses, deer and buffaloes) may also act as reservoirs. Fur-bearing animals such as mink, nutria and silver foxes as well as some reptiles and amphibians may also carry *Leptospira* (WHO, 2003).

Animals susceptible to infection with *Leptospira* are classified into two main groups: natural maintenance hosts and accidental (or incidental) hosts (WHO, 2003). Bases on definitions used in this

report, maintenance hosts can be considered as reservoir hosts, whereas accidental hosts correspond to dead-end hosts.

A natural maintenance host can be defined as an animal species in which *Leptospira* may be endemic, direct transmission usually occurs in young animals, and prevalence of chronic excretion of *Leptospira* in urine increases with age (Levett, 2001). Chronic kidney infection of maintenance hosts (renal carriers) ensures perpetuation of *Leptospira* in nature (Babudieri, 1958; WHO, 2003; Vijayachari et al., 2008). Urinary excretion of *Leptospira* may last a long time, even for the entire life of certain maintenance hosts. In farm animal maintenance hosts, such as cattle, *Leptospira* can also infect reproductive organs and cause abortions, still-births, failure to thrive and loss in milk production (Faine, 1994). However, in general, clinical and pathological consequences of infection in maintenance hosts are mild or absent. In conclustion, maintenance hosts are essential for the presence of *Leptospira* in the environment.

Conversely, accidental hosts do not play a critical role in the maintenance of *Leptospira* and are usually infected by indirect (or direct) contact with the maintenance host (Levett, 2001). Furthermore, accidental hosts may suffer severe consequences from *Leptospira* (including death) and, if they survive, the infection has a short duration in comparison with the infection of maintenance hosts (WHO, 2003).

The association between *Leptospira* serovars and animal species, and the different role that each animal species may play for different serovars are key features of the eco-epidemiology of these agents. As an example, cattle are maintenance hosts for *Leptospira hardjo*, pigs for serovar *pomona*, small rodents (including rats) for serovars *icterohaemorrhagiae*, *copenhageni*, *bataviae*, *autumnalis*, *australis* and *javanica*, dogs for *canicola* (Bolin, 2000). The same animal species may act as maintenance host for more than one serovar, and a serovar may adapt to new maintenance hosts (Hartskeerl and Terpstra, 1996). Reinfection of an animal with the same serovar rarely occurs (due to immunity), but a different serovar may cause a new infection, possibly with clinical consequences. A summary of the association of animals and *Leptospira* serovars is presented in Table 11.

Table 11. Associations between *Leptospira* serovars and animal host species (modified from Faine, 1994).

Animal species	Maintenance host	Accidental host
Rodents	*icterohaemorrhagiae, grippotyphosa, ballum*	
Cattle and deer	*hardjo*	*pomona, icterohaemorrhagiae, grippotyphosa bratislava hebdomadis, kremastos, tarassovi, autumnalis, australis, sejroe, canicola, bataviae*
Pig	*pomona, tarassovi, bratislava*	*icterohaemorrhagiae, canicola*
Sheep	*hardjo, pomona*	*grippotyphosa, bratislava.*
Dog	*canicola*	*bratislava, grippotyphosa, icterohaemorrhagiae*
Horse	*bratislava*	*pomona, sejroe, icterohaemorrhagiae, canicola,*

A general diagram of the transmission cycle of *Leptospira* is shown in Figure 5. In the diagram, transmission is represented by arrows. A distinction is made between rodent and other species of maintenance hosts. In fact, infection of rodent maintenance hosts is characterised by an almost

complete absence of clinical signs. On the contrary, other animals, such as cattle, may suffer clinical consequences even when they act as maintenance hosts.

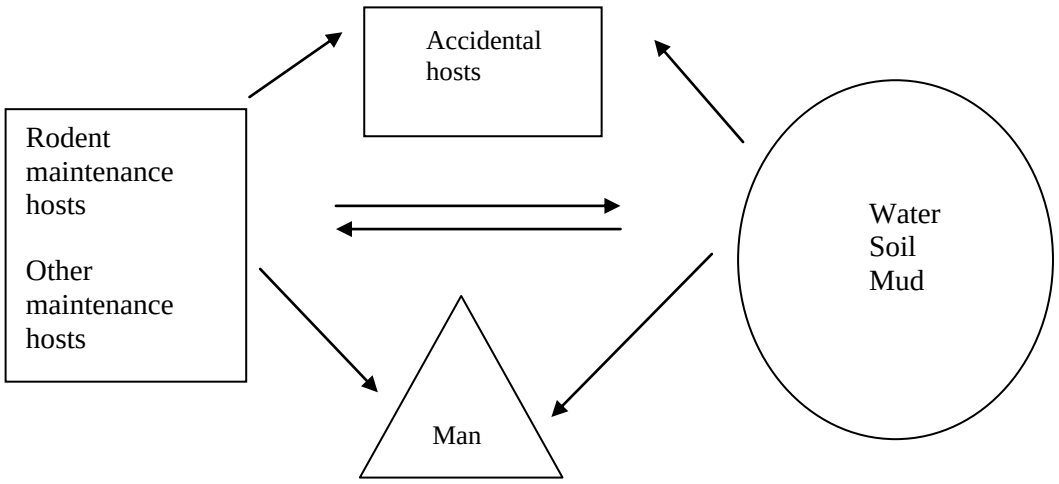

Figure 5. Diagram of the transmission of *Leptospira* with specific focus on the role of animals. See Table 11 and the text for details on the role of animal species for different *Leptospira* serovars.

3.1.2. Diagnosis of *Leptospira* in animals

Clinical signs of Leptospira in animals.

Clinical signs of leptospirosis in animals vary based on animal species and *Leptospira* serovar. In general, an acute phase, lasting approximately one week and with leptospiremia, is followed by a chronic or immune phase with leptospiruria (Levett, 2001). Acute signs of leptospirosis are mainly observed in young animals and in accidental hosts 3 – 7 days post infection, and include loss of appetite, fever, red eyes, listlessness, ruffled fur and sometimes diarrhoea and/or signs of jaundice and haemorrhages. Reduction of milk production is well known for dairy cows. Severity of signs is very variable, and subclinical forms are frequent and, therefore, leptospirosis can go undetected (WHO, 1982; Faine 1994; Vijayachari et al., 2008).

In cattle and pigs, the most relevant features of the chronic phase are reproductive failure (late abortions, premature births and stillbirths), and interstitial nephritis which may cause chronic renal failure (Bolin et al., 1991; Smith et al., 1994; Levett, 2001). A mild to severe chronic interstitial nephritis, clinically inapparent, may be detectable at necropsy or at slaughter. *Leptospira* can also remain in other organs of the host, such as the brain, the genital tract and the eye (WHO, 1982).

Laboratory diagnosis of Leptospira in animals

Direct laboratory diagnosis

The isolation of *Leptospira* is the most specific method of demonstrating its presence in tissues and body fluids, provided that antibiotic residues are absent, that tissue autolysis is not advanced, and that tissues are processed for culture rapidly after collection (Ellis, 1986).

Immunochemical staining (immunofluorescence and immunohistochemical techniques) and an antigen capture ELISA (Sharma et al., 2008) can be used to detect *Leptospira* in pathological material that is unsuitable for culture or where a rapid diagnosis is required (Bolin et al., 1989; Ellis, 1986; Scanziani et al., 1991; Wild et al., 2002). Since sensitivity of these techniques is affected by the number of

organisms present, they are less suitable for diagnosing the chronic carrier state, where the numbers of spirochetes may be very low or localised.

PCR is used in several laboratories for the detection of *Leptospira* in tissues and body fluids of animals. A variety of primer sets for PCR assays have been described (van Eys et al., 1989; Branger et al., 2005; Gravekamp et al., 1993; Hookey, 1992; Kawabata et al., 2001; Levett et al., 2005; Merien et al., 2005; Palaniappan et al., 2005; Reitstetter, 2006; Smythe et al., 2002; Mayer-Scholl et al., 2011; Rojas et al., 2010), some of them only specific for the genus *Leptospira* and others designed to identify pathogenic species.

Characterisation of *Leptospira*

The identification of isolates is a task for specialized reference laboratories. A pure leptospiral culture may be identified as belonging to a pathogenic or saprophytic species by a variety of tests, based on the ability to infect animals, biochemical and cultural features, and molecular tests (Johnson and Harris, 1967; Johnson and Faine, 1984; Faine, 1994; Woo et al., 1997, Yasuda et al., 1987). Differentiation to the serovar level is traditionally obtained by cross-agglutination absorption (Dikken and Kmety, 1978), although for most isolates the serovar / species identification is now being done using less time-consuming methods, by various molecular strategies (Ralph et al., 1995; Roy et al., 2004; Brown and Levett, 1997; De Caballero et al., 1994; Corney et al., 1993; Gerritsen et al., 1991; Ramadass et al., 1992; Perolat et al., 1994; Savio et al., 1994; Vijayachari et al., 2004; Morey et al., 2006; Ahmed et al., 2006; Salaun et al., 2006; Slack et al., 2006; Pavan et al., 2011; Galloway and Levett, 2010). However, genetic-based tests may not always give the same results as the cross-agglutination absorption test. Different isolates belonging to a single serovar usually belong to the same species, but this is not always the case.

Indirect laboratory diagnosis

IgM agglutinating antibodies against *Leptospira* appear in the serum within a few days after infection (3 to 10 days) and persist for weeks or months. However, in some cases, antibodies persist for years or even through the entire life of animals (Levett, 2001). In general, antibodies peak between 2 and 3 weeks post infection. Humoral immunity can be passively transferred by serum, via colostrums and via placenta (Faine, 1994). Currently, commercial vaccines for cattle, dogs and pigs are available. Vaccines only confer serovar-specific immunity; moreover, vaccinated animals can be infected and become carriers of the organism (Srivastava, 2006).

A wide variety of serological tests, which show varying degrees of serogroup and serovar specificity, have been described for the detection of antibodies against *Leptospira*. In animals, the microscopic agglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA) are the most commonly used.

MAT using live antigens is the most widely used serological test. It is the reference test against which all other serological tests are evaluated and is used for import/export testing. For optimum sensitivity, it should use antigens representative of all the serogroups known to exist in the region in which the animals are found. The sensitivity of the test can be improved by using local isolates rather than reference strains, but reference strains assist in the interpretation of results between laboratories. There is significant serological cross-reactivity between serovars and serogroups of *Leptospira* and an animal infected with one serovar is likely to have antibodies against cross-reacting serovars (usually at a lower titre level). In addition, animals that have been vaccinated against leptospirosis may have antibodies against the serovars present in the vaccine used. Therefore, it is particularly important to consider the vaccination history of the animals under test. The result of the test may be reported as the endpoint dilution of serum (e.g. 1/100 or 1/400) (Faine et al., 2000). MAT is useful in diagnosing acute infection; the demonstration of a four-fold change in antibody titres in paired acute and convalescent serum samples is recomended. A consistent clinical picture enhances the positive predictive value. The test has limited sensitivity in diagnosis of chronic infection, both in the diagnosis

of causes of abortion and in the identification of renal or genital carriers (Ellis, 1986). This is particularly true for infection of cattle by the serovar *hardjo*, or the infection of pigs with serovar *bratislava*. As leptospirosis is a herd problem, in farm animals the MAT has much greater use as a herd test (Cole et al., 1980).

ELISAs for detection of anti-leptospiral antibodies have been developed using a number of different antigens and assay protocols. In general, ELISAs are quite sensitive, but lack the serovar specificity of the MAT. An ELISA that measures canine IgG and IgM against various leptospiral serovars has been developed and evaluated in Europe (Hartmann et al, 1986). A rapid test for the serodiagnosis of acute canine leptospirosis, that detects IgM antibodies has been recently developed (Abdoel et al., 2011). ELISA to detect anti-leptospiral antibodies in bovine, ovine and porcine serum or for multiple species have also been developed (Bomfim et al., 2005; Mariya et al., 2006; Sankar et al., 2010; Dey et al., 2004; Dey et al., 2007; Okuda et al., 2005; Palaniappan et al., 2004; Iwamoto et al., 2009; Naito et al., 2007; Wasiński and Pejsak, 2010; Theodoridis et al., 2005), but these tests are not internationally standardized and widely available. ELISAs have also been developed for use in milk, for the detection of serovar *hardjo* antibodies from individual cows or in bulk tank milk to estimate the herd prevalence (Lewis et al., 2009).

3.1.3. Data collection on *Leptospira* in animals by competent authorities

Summary of results of questionnaires submitted to the Task Force on zoonoses data collection
Twelve MSs (Denmark, Estonia, Finland, France, Hungary, Italy, Latvia, Portugal, Romania, Slovakia, Slovenia, Sweden) and Norway and Switzerland responded to the questionnaires regarding *Leptospira*.

Leptospira is notifiable in animals in Denmark, Estonia, Finland, Italy, Latvia, Norway, Romania, Slovenia, Sweden, and Switzerland.

In Finland, Romania, Sweden and Switzerland both active and passive data collection are carried out. In Estonia, only active surveillance is performed. In Denmark, France, Hungary, Italy, Latvia, Norway, Slovakia, Slovenia only passive surveillance in carried out. In Portugal no data collection activity (active/passive) in animals is performed. Summaries of active and passive data collections are presented in Table 12. Detailed information provided by individual countries is presented in Annex A.

Table 12. Summary of results of questionnaire on data collection on *Leptospira* in animals by competent authorities[a]

Country	Active data collection			Passive surveillance			National Reference Laboratory
	Time span	*Animal species*	*Diagnosis*	*Time span*	*Animal species*	*Diagnosis*	
Denmark					Pig, cattle	MAT	National Veterinary Institute, Technical University of Denmark
Estonia	2004-present	Cattle	Indirect ELISA				Estonian Veterinary and Food Board
Finland	1980-2009	Cattle, pigs	MAT	1980-2009 2005-2009	Pig, cattle Dog	MAT PCR	Finnish Food Safety Authority
France		Dog	MAT				Centre National de Référence (CNR) de la leptospirose, Pasteur Institute.
Hungary					Pig, cattle, equids, dogs	MAT, histology, IHC	Central Agricultural Office, Veterinary Diagnostic Directorate
Italy				1995-2010	Dogs, equids, cattle, pigs sheep, wild boars, rodents	MAT	Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna
Latvia				1999-present		MAT	Institute of Food Safety, Animal Health and Environment
Norway				1990-present	Pigs dogs	MAT	Norwegian Food Safety Authority
Portugal							Laboratório Nacional de Investigação Veterinária.
Romania	1980-present	Cattle, pigs, equids, dogs	MAT	1980-present	Cattle, pigs, equids, dogs		Central Veterinary Authority
Slovakia				1980 – 2009	Dogs, cattle, pigs, rodent, sheep, deer, wild boars, goats, equids	MAT	State Veterinary and Food Institute
Slovenia					Cattle, pigs, dogs, equids, sheep, goats, rodents, deer, wild boars	MAT	Veterinary Administration of Republic of Slovenia
Sweden	2005-2007	Rodents				MAT, ELISA	National Veterinary Institute
		Pigs, wild, boars	MAT				
Switzerland	2006-2008	Equids	MAT			MAT	University of Bern, Vetsuisse Faculty

(a) MAT: microscopic agglutination test, IHC: immunohystochemistry.

3.1.4. Systematic literature review on data collection on *Leptospira* in animals

The literature search was carried out on publications from 2000 through 2010, with the exception of a single article from Latvia that was published in 1998. Results by country, information on diagnostic tests and sampling are presented in Annex B.

Results of active data collection on Leptospira by animal species

Main results of active data collection are summarised in Table 13. Forty six publications were used in prevalence calculation, from 20 countries: Austria, Bulgaria, Czech Republic, Denmark, France, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, United Kingdom. One publication reported a study on multiple countries, including Germany and Luxemburg (see Annex B for details).

There was great variability across species and, as expected, greatest prevalence levels were found in rodents (with the exception of a single study on two badgers). Domestic animals are characterised by low prevalence, and this might be due to low levels of exposure to *Leptospira*, but also to variable duration of antibody response in these species.

In the interpretation of these results, however, is it to be taken into account that substantial discrepancies were found among the retrieved articles regarding serological tests. In particular, the definition of negative or positive results from the MAT was not harmonized among the studies. As an example, cut-off levels for considering a sample positive ranged from titres ≥ 1:10 to ≥ 1:800. In general, the absence of relevant articles in a country (based on the systematic literature review) does not imply absence of *Leptospira*.

Because of the ecological and epidemiological relevance of the association between animal species and *Leptospira* serovars, the findings of each serovar are presented, by animal species, in Table 15.

Table 13. Results of active data collection on *Leptospira* in animal species based on the systematic review of publications in the EU, Norway and Switzerland, from 2000 through 2010[(a)].

Species	N of countries	N of studies	N of tested animals	N of positive animals	Prevalence	Lower 95% CL	Upper 95% CL
Cattle[(b)]	9	13	21529	1274	5.92	3.41	10.09
Dog[(c)]	8	8	6670	781	11.71	4.90	25.46
Horse[(d)]	7	8	17322	1387	8.01	2.93	20.04
Wild boar[(e)]	5	15	2457	364	14.81	5.91	32.50
Rat[(f)]	5	12	390	169	43.33	28.39	59.60
Pig[(g)]	5	9	6038	542	8.98	6.28	12.68
Sheep[(h)]	4	15	2852	211	7.40	4.11	12.97
Wild mice[(i)]	3	11	557	72	12.93	9.55	17.27
Nutria[(j)]	3	5	1867	548	29.35	15.16	49.14
Deer[(k)]	2	15	907	76	8.38	5.49	12.59
Hare[(l)]	2	4	988	138	13.97	7.35	24.93
Fox[(m)]	2	3	279	28	10.04	4.64	20.38
Shrew[(n)]	2	3	37	4	10.81	2.35	37.91
Goat[(o)]	2	2	302	42	13.91	9.06	20.77
Cat[(p)]	2	2	143	39	27.27	14.06	46.22
Mus[(q)]	2	2	95	39	41.05	3.05	93.91
Genet, mangoose[(r)]	1	2	54	9	16.67	10.60	25.22
Game animal[(s)]	1	1	745	83	11.14	9.07	13.61
Rattus, mus[(t)]	1	1	244	72	29.51	24.12	35.53
Lynx[(u)]	1	1	22	7	31.82	15.99	53.37
Badger[(v)]	1	1	2	1	50.00	5.89	94.11

(a)Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study. (b) Alonso-Andicoberry et al., 2001, Arsene and Arsene, 2007, Atxaerandio et al., 2005, Burriel et al., 2003, Cerri et al., 2003, Espi et al., 2000, Krawczyk, 2005, Lewis et al., 2009, Treml et al., 2007, van Schaik et al., 2002, Vitale et al., 2005; (c) Akerstedt et al., 2010, Burriel et al., 2003, Cătană et al., 2006, Cerri et al., 2003, Khalacheva et al., 2000, Krawczyk, 2005, Millán et al., 2009, Rojas et al., 2010 ; (d) Baverud et al., 2009, Cerri et al., 2003, DEFRA, 2007, Hartskeerl et al., 2004, Platon and Vasiu, 2009b, Rocha et al., 2004, Szeredi et al., 2006; (e) Cerri et al., 2003, Ebani et al., 2003, Espi et al., 2010, Krawczyk, 2005, Montagnaro et al., 2010, Treml et al., 2003a, Vengust et al., 2008, Vicente et al., 2002 ; (f) Aviat et al., 2009, Burriel et al., 2008, Collares-Pereira et al., 2000, Krojgaard et al., 2009, Pezzella et al., 2004; (g) Alexopoulos et al., 2003, Burriel et al., 2003, Cerri et al., 2003, Krawczyk, 2005, On et al., 2002, Treml et al., 2007, Vitale et al., 2005; (h) Burriel et al., 2003, Cerri et al., 2003, Krawczyk, 2005, Spilovska et al., 2009, Vitale et al., 2005; (i) Adler et al., 2002, Aviat et al., 2009, Treml et al., 2002; (j) Aviat et al., 2009, Bollo et al., 2003, Kik et al., 2006, Michel et al., 2001; (k) Cerri et al., 2003, Espi et al., 2010; (l) Treml et al., 2003b, Winkelmayer et al., 2005; (m) Akerstedt et al., 2010, Millán et al., 2009; (n) Adler et al., 2002, Treml et al., 2002; (o) Burriel et al., 2003, Krawczyk, 2005; (p) Millán et al., 2009, Mylonakis et al., 2005; (q) Treml et al., 2002; (r) Millán et al., 2009; (s) Zitek and Babička, 2000; (t) Vitale et al., 2007; (u) Millán et al., 2009; (v) Millán et al., 2009.

Results of passive data collection on Leptospira by animal species

Results of passive data collection on *Leptospira* in animals are summarised in Table 14. Fourteen publications were retrieved from eight countries. In order to provide information on serovar distribution, animal species is included in this table.

Table 14. Results of passive data collection on *Leptospira* in animal species, based on the systematic review of publications in the EU, Norway and Switzerland, from 2000 through 2010.

Country	Animal species	N of suspected cases	N of confirmed cases	Identified serovars
France[(a)]	Horse	1	1	NA
Germany[(b)]	Dog	50	37	bratislava, grippotyphosa, pomona
Italy[(c)]	Water buffalo	80	22	NA
Italy[(d)]	Pig	20	19	NA
Latvia[(e)]	Dog	1180	211	NA
Netherlands[(f)]	Dog	98	0	NA
Netherlands[(g)]	Harbor seal	5	2	NA
Romania[(h)]	Dog	142	82	canicola, icterohaemorrhagiae, hardjo, grippotyphosa, pomona, tarassovi
Romania[(i)]	Dog	8	8	canicola
Romania[(j)]	Pig	685	129	NA
Spain[(k)]	Cattle	144	8	bratislava, hardjo
Spain[(l)]	Cattle	442	81	bratislava, canicola, icterohaemorrhagiae, ballum, grippotyphosa, tarassovi
Spain[(m)]	Pig	29	20	bratislava
United Kingdom[(n)]	Horse	42	2	NA

(a) Leon et al., 2006; (b) Kohn et al., 2010; (c) Marianelli et al., 2007; (d) Radaelli et al., 2009; (e) Peneze et al., 1998; (f) Boomkens et al., 2005; (g) Kik et al. 2006; (h) Ghiorghiţă and Perianu, 2009; (i) Ivana et al., 2008; (j) Platon and Vasiu, 2009a; (k) Atxaerandio et al., 2005; (l) Guitian et al., 2001; (m) Martinez et al. 2006; (n) Whitwell et al., 2009; NA: not available.

3.1.5. Information on the occurrence and geographical distribution of *Leptospira*, and animal hosts.

Agent

Reporting of the most common serogroups in some EU countries were published in 1999 by the International Leptospirosis Society (ILS) in collaboration with the WHO/FAO Collaborating Centre for Reference and Research on Leptospirosis (Western Pacific Region), and can be found in WHO (1999).

Based on results of the systematic literature review from 2000 through 2010, the geographic distribution of *Leptospira* serovars, that were detected in each country by active data collection, are reported in Table 15, together with animal species in which serovar characterisation was carried out. The geographic distribution of serovars, by country and animal species, that resulted by passive data collection is summarised in Table 14. It is very important to consider that information presented in this table is crucially affected by sampling on different animal species. *Leptospira icterohaemarragiae* was the most commonly reported serovar, suggesting a key role of rodents (especially rats) in the transmission of this agent to other animal species. *Bratislava* and *grippotyphosa* are other serovars widespread across countries and species. Interestingly, *Leptospira canicola* was often found in spite of the common vaccination of dogs that are maintenance hosts for this serovar.

Table 15. Geographic distribution of *Leptospira* serovars detected by active data collection in animals, based on the systematic literature review of publications from 2000 through 2010[(a)].

Country	Animal species	Identified serovars
Austria	Hare[(b)]	grippotyphosa
Czech Republic	Hare[(c)]	grippotyphosa
France	Nutria[(d)], Rat[(e)], Wild mice[(f)]	grippotyphosa, icterohaemorrhagiae
Greece	Cat[(g)], Cattle[(h)], Dog[(i)], Goat[(j)], Pig[(k)], Sheep[(l)]	canicola, ballum, bratislava, icterohaemorrhagiae, hardjo, pomona, tarassovi, grippotyphosa
Italy	Nutria[(m)], Wild boar[(n)]	bratislava, canicola, icterohaemorrhagiae, tarassovi
Netherlands	Cattle[(o)]	hardjo
Norway	Dog[(p)], Fox[(q)]	icterohaemorrhagiae
Portugal	Horse[(r)] , *Mus* spp. [(s)], Rat[(t)]	interrogans, kirschneri, ballum, icterohaemorrhagiae
Romania	Dog[(u)]	canicola, icterohaemorrhagiae, pomona
Spain	Badger[(v)], Carnivores[(w)], Cat[(x)], Cattle[(y)], Deer[(z)], Dog[(aa)], Fox[(ab)], Lynx[(ac)], Wild boar[(ad)]	icterohaemorrhagiae, ballum, bratislava , canicola, hardjo, grippotyphosa, pomona, tarassovi
Sweden	Horse[(ae)]	bratislava, icterohaemorrhagiae, grippotyphosa, pomona,
United Kingdom	Cattle[(af)]	hardjo

(a) The information presented in this table is affected by sampling different animal species and, therefore, cannot be considered as a valid estimate of the geographic distribution of serovars. (b) Winkelmayer et al., 2005; (c) Treml et al., 2003b; (d) Aviat et al., 2009; (e) Aviat et al., 2009; (f) Aviat et al., 2009; (g) Mylonakis et al., 2005; (h) Burriel et al., 2003; (i) Burriel et al., 2003; (j) Burriel et al., 2003; (k) Alexopoulos et al., 2003, Burriel et al., 2003; (l) Burriel et al., 2003; (m) Bollo et al., 2003; (n) Ebani et al., 2003, Montagnaro et al., 2010; (o) van Schaik et al., 2002; (p) Akerstedt et al., 2010; (q) Akerstedt et al., 2010; (r) Rocha et al., 2004; (s) Collares-Pereira et al., 2000; (t) Collares-Pereira et al., 2000; (u) Cătană et al., 2006; (v) Millán et al., 2009; (w) Millán et al., 2009; (x) Millán et al., 2009; (y) Alonso-Andicoberry et al., 2001, Atxaerandio et al., 2005, Espi et al., 2000; (z) Espi et al., 2010; (aa) Millán et al., 2009; (ab) Millán et al., 2009; (ac) Millán et al., 2009; (ad) Espi et al., 2010, Vicente et al., 2002; (ae) Baverud et al., 2009; (af) Lewis et al., 2009.

Hosts

For the distribution of animal hosts for *Leptospira*, see Annex F.

3.2. Proposal for data collection on *Leptospira* in animals

The application of criteria for the selection of animal species to be proposed for data collection can be summarised as follows (the application of criteria can be seen in detail in Annex C).

The selection of animal species for data collection on *Leptospira* is based upon the the role of the species as a source of infection for the environment and for humans, practical feasibility of data collection, the current existence of data collection activities on *Leptospira* and for other agents.

Synanthropic rodents and especially rats (*Rattus* spp.) are the most common source of *Leptospira* through contamination of water and indirect transmission to humans.

Other domestic and wild animals may contribute to contamination of water and cause human infection during occupational and recreational activities. Occupational leptospirosis is reported for slaughterhouse workers and farmer, caused by *L. pomona* e *L. bratislava* (carried by pigs) and *L. hardjo* (carried by cattle).

The role of dogs in transmission of *Leptospira* to humans is uncertain. Nevertheless, dogs are included in data collection given the close contact and the sharing of environment of this species with man, and its susceptibility to *Leptospira*.

Active data collection on *Leptospira* is proposed for rats and other rodents, dogs (where *Leptospira* is re-emerging), as well as for wild boars and other wild animals (foxes) during hunting in order to detect occurrence and trends of *Leptospira* in the environment (Millán et al., 2009, Muller and Winkler, 1994). For some domestic species, such as cattle, active data collection is not proposed due to short duration or intermittent pattern of antibody response.

Based upon questionnaire to the Task Force on Zoonoses data collection, passive data collection on *Leptospira* in animals is currently carried out in most countries (Table 12). Passive data collection, based on the identification of suspected cases and subsequent laboratory confirmation, is to be recommended in all countries, in domestic and synanthropic animal. These species can act as sources of infection for humans or allow the detection of the risk of transmission of *Leptospira* from other animal sources.

Animal species living in close contact with humans and are the object of veterinary activities are particularly useful, since clinical signs and pathological lesions can be easily detected.

Health status of farmed species is constantly monitored, and reproductive disorders, such as abortion, are followed by investigations for other agents relevant for public health (i.e. brucellosis).

Food animals are regularly inspected during slaughter, and lesions that may be caused by *Leptospira* (i.e. chronic interstitial nephritis of pigs) can be detected.

Based on the above, we conclude that all suspect cases of *Leptospira* in animals should be submitted for laboratory confirmation in a passive data collection system.

Proposed animal species and type of data collection (active, passive) are listed in Table 16 Specifications for data collection on each animal species are described below, and summarised in Table 17 and Table 18.

Table 16. List of animal species and type of data collection proposed on *Leptospira*.

Species	Type of data collection	
	Passive	**Active**
Rodents	-	X
Dog	X	X
Cattle	X	-
Other farmed ruminants, including deer	X	-
Pigs and farmed wild boars	X	-
Wild boars	-	X
Horses	X	-
Wild carnivores	-	X

3.2.1. Data collection on *Leptospira* in rodents

Specifications for active data collection on Leptospira in rodents

Subpopulations to be covered

Synanthropic (*Rattus* spp., *M. musculus*) and wild rodents (*Apodemus* spp., *Myodes glareouls*, *Microtus* spp.) can be considered in active data collection.

Agents and possible subtypes

All *Leptospira* serovars should be considered given the potential threat for public health.

Sampling context and design, sampling stage.

Rodents should be trapped in urban and rural environment that are frequented by people and domestic animals. In active data collection on wild rodents, land areas should be the primary sampling units. Accordingly, each country could be divided into 20 by 20 km squares. One square should then be randomly selected in each first order administrative area, retaining only those including wooded areas frequented by people. Availability of personnel in protected areas could also be a factor in the inclusion of locations for data collection. Rodents should be trapped overnight by using live traps, during monthly sessions in spring and summer (wild rodents are mostly active in these seasons, moreover live trapping in cold seasons may lead to death of animals).

For data collection on synanthropic rodents, such as rats, trapping sites should be identified in rural areas (near farms) and in urban and suburban areas.

Sampling design should rely on stratification at the level of the first order administrative area in each country. In practice, rodent traps should be placed at selected locations, in order to enhance representativity and precision of prevalence estimats. In fact, rodent population density and *Leptospira* prevalence vary across locations.

Type of specimen to be taken and sampling technique

Blood serum and kidney from sacrificed rodents are to be collected.

Diagnostic methods to be used

PCR on kidney and MAT on serum using a 1:100 cut off (Faine et al., 2000).

Data submission

Information on season, geographic location, and frequency (i.e. number of trap-nights) of trapping should be reported.

3.2.2. Data collection on Leptospira in dogs

Specifications for active data collection on Leptospira in dogs

Subpopulations to be covered

Hunting, shepherd dogs, and dogs frequenting outdoor environment should be considered. Shelter dogs can also be included if sampled immediately after capture to eliminate the effect of *Leptospira* transmission within shelters.

Agents and possible subtypes

All *Leptospira* servovars should be considered given the potential threat for public health.

Sampling context and design, sampling stage

Realistically, a convenience sampling of dogs, based upon owner compliance is to be carried out.

Type of specimen to be taken and sampling technique

Blood serum.

Diagnostic methods to be used

MAT should be used for demonstration of antibodies against *Leptospira*. MAT cut off antibodies' titre should be set as follows: 1:100 for unvaccinated dogs or for vaccinated dogs against the serovars not included in the vaccine; 1:800 for vaccinated dogs and for the serovars included in the vaccine.

Specifications for passive data collection on Leptospira in dogs

Subpopulations to be covered

All dogs can be considered in passive data collection, although those animals frequenting outdoor environments are most likely to be infected with *Leptospira*.

Agents and possible subtypes

All *Leptospira* servovars should be considered given the potential threat for public health.

Sampling context and design, sampling stage

In passive data collection, criteria for defining suspect cases are crucial. The following forms of disease can be caused by *Leptospira* in dogs:

Peracute disease (rare), with death, following short-lasting signs of shock.

Acute disease (uncommon), characterised by pyrexia, muscle weakness, endotoxic shock with coagulopathy and vascular injury, death.

Subacute disease (the most common form) with fever, mucosal petechiation; anorexia; vomiting, polyuria, polydipsia, sometimes anuria; jaundice; sometimes diarrhea; reluctance to move.

Chronic (uncommon): jaundice with chronic active hepatitis; anterior uveitis.

The observation of the above signs should be considered for subsequent laboratory confirmation.

Type of specimen to be taken and sampling technique
In dead animals, lung, liver, kidney should be collected. Blood serum should be collected from live dogs.

Diagnostic methods to be used

Confirmation should be based on demonstration of *Leptospira* in the organs (lung, liver, kidney) by culture, PCR or IHC, or demonstration of antibodies by MAT (a specific rise of titre can be observed between acute and convalescent serum). Specific IgM can be detected by ELISA.

3.2.3. Data collection on *Leptospira* in wild boars and wild carnivores

Specifications for active data collection on Leptospira in wild boars and wild carnivores (foxes)
Subpopulations to be covered

Wild animals from areas frequented by humans should be considered in active data collection.

Agents and possible subtypes

All *Leptospira* serovars should be considered given the potential threat for public health.

Sampling context and design, sampling stage

Sampling should be based on the hunting activity. An effort should be made to have a stratified sample by first order administrative areas.

Type of specimen to be taken and sampling technique

Blood serum and kidney are to be collected.

Diagnostic methods to be used

PCR on kidney and MAT on serum (cut off 1:100).

Data submission

Information on season of hunting and wild animal population in each administrative area should be reported if available.

3.2.4. Data colletion on *Leptospira* in ruminants

Specifications for passive data collection on Leptospira in ruminants
Subpopulations to be covered

All ruminants should be considered in passive data collection

Agents and possible subtypes

All *Leptospira* serovars should be considered given the potential threat for public health.

Sampling context and design, sampling stage

The following clinical signs should be considered in defining a supect case of *Leptospira* in ruminants: jaundice, hematuria, hemoblobinuria, evidence of renal damage, meningitis. In cows and ewes, the following clinical signs are to be considered: abortion or delivery of a premature/stillborn offspring, especially when preceded by a mastitis with rapid loss of milk production.

Type of specimen to be taken and sampling technique.
Organs, such as kidney, lung, liver should be collected from dead animals and aborted fetuses, and blood serum from live animals. Serum should be collected twice for paired testing to detect titre raise. After abortion of an animal in a herd or flock, all females having showed reproduction disorders

(including stillbirth) in the previous 60 days should be sampled, together with other animals that showed no reproductive sign.

Diagnostic methods to be used.
Culture, PCR or IHC on organs, MAT on blood serum.

3.2.5. Data collection on *Leptospira* in pigs.

Specifications for passive data collection on Leptospira in pigs.

Subpopulations to be covered
Breeding pigs (at farm) and slaughter pigs (at slaughter)

Sampling context and design, sampling stage
Suspect cases of leptospirosis in pigs should be based on clinical/pathological observations at farm and at slaughter. At farm, criteria include reproductive disorders and late pregnancy abortion, stillbirth, premature births. At slaughter, suspicion of infection by *Leptospira* should be raised by detection of chronic interstitial nephritis (white spotted kidneys).

Type of specimen to be taken and sampling technique

At farm, specimen of abortion materials (kidney, lung or liver of foetus) and blood serum in the involved sow and in healthy sows for comparison. At slaughter, kidney showing white spots, and serum sample from pigs of the same slaughter lot (when possible).

Diagnostic methods to be used

Culture, PCR or IHC on organs, MAT for antibody detection in blood serum.

Data submission

Number of pigs in farm, and in slaughter lot should be reported, to allow to take into account clustering of observations at the farm and at the lot level, and to include these information to obtain adjusted prevalence estimates.

3.2.6. Data collection on *Leptospira* in horses.

Specifications for passive data collection on Leptospira in horses.

Subpopulations to be covered

All horses should be considered.

Agents and possible subtypes

All serovars.

Sampling context and design, sampling stage

Suspect cases should be animals showing abortion or recurrent uveitis.

Type of specimen to be taken and sampling technique

Kidney, lung or liver of aborted foetus, ocular fluid in case of recurrent uveitis.

Diagnostic methods to be used
Culture, PCR or IHC on organs of foetus, Culture, PCR on ocular fluid.

Table 17. Summary of proposals for active data collection on *Leptospira* in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimes	Diagnosis
Synanthropic and wild rodents	Populations in urban and rural areas	Live trapping and euthanasia	In 20 x 20 km squares stratified by administrative areas	Blood serum, kidneys	PCR on kidneys, MAT on serum, cut off 1:100
Dogs	Dogs frequenting outdoor environment	Convenience sampling based on owner compliance	Stratified by administrative areas	Blood serum	MAT, cut off 1:100 if unvaccinated, 1:800 if vaccinated
Wild boars and wild carnivores	Populations from areas frequented by humans	Convenience sampling based on hunting	Stratified by administrative areas	Blood serum, kidneys	PCR on kidneys, MAT on serum, cut off 1:100

Table 18. Summary of proposals for passive data collection on *Leptospira* in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Type of specimes	Diagnosis
Dog	All dogs	See criteria for suspect cases, reported by owners and veterinarians	Blood serum, lung, liver, kidneys	MAT and IgM-ELISA on serum, bacterial culture, PCR, IHC on organs
Ruminants	All ruminants		Blood serum, lung, liver, kidneys, aborted foetuses	
Pigs	Breeding pigs	At farm, criteria: reproductive disorders	Blood serum, kidney, lung or liver of foetus	
	Slaughter pigs	At slaughter: white spot kidneys	Blood serum, kidneys	
Horses	All horses	Criteria: abortion, recurrent uveitis	Kidney, lung or liver of aborted foetus, ocular fluid in uveitis	Culture, PCR or IHC on organs of foetus; culture, PCR on ocular fluid

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4. WEST NILE VIRUS

West Nile virus (WNV) belongs to the *Flavivirus* genus in the family *Flaviviridae* (Japanese encephalitis antigenic complex). In man, WNV most commonly causes asymptomatic or mild febrile forms, whereas in a small fraction of cases (~1%), it causes meningitis and encephalitis.

4.1. Inventory of available data on WNV in animals

4.1.1. Transmission cycle of WNV

WNV is maintained in transmission cycles involving bird reservoir hosts and mosquito vectors. In Europe, two types of transmission cycles can be distingushed: 1) rural/sylvatic cycle, involving wild birds and ornithophilic mosquitoes; b) urban cycle, involving synanthropic or domestic birds and mosquitoes feeding on both birds and humans (Komar 2000). Humans, horses and other mammals are considered as dead-end hosts. Humans can also be potentially infected through organ transplantation, blood transfusion; trans-placental transmission and iatrogenic or accidental inoculation are also reported.

Culex (Cx.) *pipiens* and *Cx.modestus* are the main vectors of WNV in the EU (Hubálek and Halouzka, 1999; Calzolari et al., 2010a, Calzolari et al., 2010b, Almeida et al., 2008, Vazquez et al., 2010; Hubálek et al., 2000; Savage et al., 1999; Hannoun et al., 1964; Balenghien et al., 2008). *Cx. pipiens* is one of the most common and widespread Holarctic mosquitoes, with a distribution covering all temperate regions. *Cx. modestus* is commonly found in delta regions, lakes, and ponds, widespread in Europe. The role of *Cx. perexiguus* should be also considered in southern Europe (Almeida et al., 2008).

In bird species acting as reservoir/amplifying hosts, viremia lasts 1-7 days post infection (based on variations across species). During this period, birds are able to transmit WNV to susceptible mosquitoes and, subsequently, develop life-long immunity.

Based on the duration of viraemia, reservoir hosts can be classified as highly competent hosts (HCH) and mildly competent hosts (MCH) (Komar et al., 2003; Castillo-Olivares and Wood, 2004). In laboratory studies, birds belonging to the orders *Passeriformes*, *Charadriiformes*, *Strigiformes*, and *Falconiformes* developed levels of viremia that were sufficient to infect most of the feeding mosquitoes. Conversely, viremia levels observed in *Columbiformes*, *Piciformes*, and *Anseriformes* have been shown as insufficient to infect feeding mosquitoes (Hayes et al., 2005). Domestic *Galliformes*, such as chickens and turkeys, develop viremia, although susceptibility decreases rapidly with age and these species develop high-titre antibody responses (Savage et al., 1999). Moreover, viremia in turkeys seems insufficient to cause infection of mosquitoes. However, the use of sentinel chickens is known to be a cost-effective and non invasive alternative to the use of wildlife species for detecting WNV circulation and is used frequently to monitor the presence of this virus (Buckley et al., 2006; Rizzoli et al., 2007).

Migratory birds may introduce the virus from endemic areas (such as, for example, overwinter African regions) into new territories. Resident birds may, subsequently, act as amplifying hosts (Autorino et al., 2002; Linke et al., 2007). Favorable condition for WNV introduction and amplification can be found in wetlands, for the simultaneous presence of migratory and resident birds, as well as abundant mosquito populations (Jourdain et al., 2007a). During late summer, birds may spread WNV from wetland to surrounding areas. The role of migratory birds in WNV spreading has been clearly assessed by several studies in European countries, including Spain (Figuerola et al., 2007), Portugal (Esteves et al., 2005), Poland (Hubálek et al., 2008), Romania and Czech Republic (Calistri et al., 2010). Infections of migratory birds with WNV can occur either through exposure on migratory routes in Europe, or at wintering site in Africa.

The possibility of WNV to overwinter in Europe is not completely known, and field observations lead to somewhat contradictory conclusions. In fact, the sporadic outbreaks in Southern Europe (Romania, Tuscany-Italy, Camargue-France) do not support the assumption that WNV may overwinter in these regions, but indicate that WNV is occasionally imported (Linke et al., 2007). On the other hand, the recent observation of a few cases of human diseases for a limited number of seasons after an outbreak (Hubálek et al., 2000), and biomolecular data (Rossini et al., 2011, Calzolari et al., 2010b) suggest the ability of WNV to overwinter in Europe, and a possible endemisation of this virus (Calistri et al., 2010). Furthermore, the review of recent outbreaks in Europe seems to support the overwintering hypothesis (Chaskopoulou et al., 2011).

The epidemiological characteristics of WNV epidemics in Italy are very interesting. In fact, synanthropic, resident birds, such as *Corvidae* (*Corvus cornix*, *Corvus monedula*, *Pica pica*, *Garrulus glandarius*) and also pigeons (*Columba livia*) and stirlings (Sturnus vulgaris) were virologically positive in Italy in 2008 (Calistri et al., 2010). Moreover, the re-occurrence of WNV transmission in 2009 in areas far from localities with a high density of migratory birds, and positive virological results on resident birds, may suggest the establishment of an efficient local overwintering mechanism with the possible involvement of these bird species. The real role of these findings as a evidence of the maintenance of the infection during interepizootic periods needs to be clarified. Indeed, wild synanthropic birds were suspected to be amplifying hosts in the WNV epizootic in France as well (Jourdain et al., 2007a).
The transmission cycle of WNV, with emphasis on the role of animals, is represented in a diagram in Figure 6. Transmission of WNV (represented by arrows) may occur between vectors and bird reservoir hosts (arrows indicate transmission in both directions, from, and to reservoir hosts and vectors). Vectors can also transmit WNV to dead-end hosts. Among these animal species, clinical disease may occur in horse and man. Other dead-end hosts include vertebrates such as ruminants, which serve as hosts for mosquitoes and may develop antibody response against WNV, but do not show clinical signs. Those bird species where viremia is not sufficient to infect feeding mosquitoes may also be included in the "other dead-end hosts" category. This classification of animals involved in WNV transmission cycle will be used as a basis for the selection of animals to be included in proposals for data collection (see below).

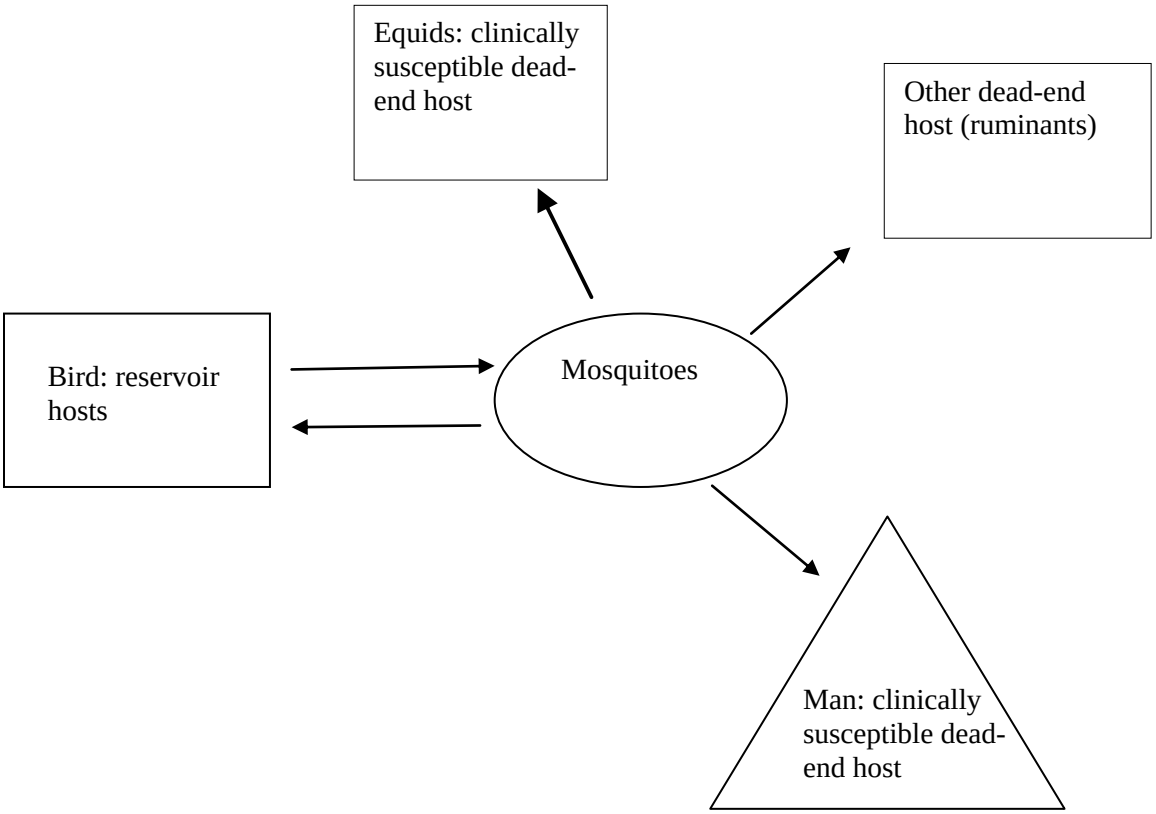

Figure 6. Diagram of the transmission of WNV with specific focus on the role of animals.

4.1.2. Diagnosis of WNV in animals

Clinical signs of WNV in animals

In Europe, clinical signs of WNV are mostly seen in horses. Approximately 10% of horses infected with WNV present neurological disorders (Komar et al, 2003). In Europe, birds mortality related to WNV infection is rare (unlike in North America). European birds usually do not show any symptoms when infected, which is taken to indicate that the virus has been circulating amongst both migrant and resident birds for many years (Gould, 2003), producing herd immunity or selecting the more resistant individuals. WNV infection in symptomatic birds was confirmed, in Hungary, in domestic goose (*Anser anser*) showing ataxia and other neurological signs (Glávits et al., 2005), and in goshawks (*Accipiter gentilis*) and sparrow hawks (*Accipiter nisus*) in a rehabilitation centre (Erdélyi et al., 2007). WNV clinical disease, with mortality, was also reported in the red-legged partridge (*Alectoris rufa*) in an experimental infection (Sotelo et al., 2011).

Laboratory diagnosis of WNV in animals

Laboratory diagnosis of WNV infections in animals, could be conducted either by direct or indirect methods.

Direct laboratory diagnosis

Viral nucleic acid can be demonstrated in tissues of infected animals by reverse-transcription polymerase chain reaction (RT-PCR), and viral antigens can be detected by immunohistochemistry. Virus isolation in cell cultures can also be used.

Several RT-PCR protocols have been described for detection of WNV RNA (Kauffman et al. 2003). PCR protocols can be used on a variety of sample types, including clinical samples such as cerebrospinal fluid, and post mortem tissue samples. Both the standard RT-PCR and the Real Time RT-PCR are characterized by high specificity and sensitivity. Multiplex Real time RT-PCR (TaqMan PCR) is rapid and allows saving materials, but there is a loss in sensitivity. The PCR amplicons can be sequenced, providing important epidemiological data such as the lineage and the identity between the different detected strains.

Virus isolation is the gold standard for viral detection, but it requires long time compared with other techniques, and this is a severe limit.

Viraemia in horses is characterized by short duration and low titre, therefore a high level of detection is possible in a short period in the early stage of infection only and this must be kept in mind while considering RT-PCR methods.

Immunocytochemistry (IHC) on central nervous sytem (including cortex, cerebellum, brain stem and spinal cord) using WN-specific monoclonal antibodies (MAb), can be used (Cantile et al., 2001). Approximately 50% of equine with WN viral encephalitis yield negative results by IHC, as a consequence, failure to identify WNV antigen in equine central nervous system, by IHC, does not rule out infection.

Indirect laboratory diagnosis

Indirect methods include IgG and/or IgM antibody detection in blood, plasma or serum samples by enzyme linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), indirect hemoagglutination test (IHA), complement fixation and seroneutralization tests.

IgMs are detected 2-8 days after onset of clinical signs (if present), or 6–7 days after infection, peak at 2 weeks post infection and then decrease over several weeks or months. IgGs appear a few days after IgM and may persist for years (Dauphin and Zientara, 2007). As a severe drawback, indirect tests are unable to differentiate between natural infection and seroconversion due to vaccination (information to be considered especially for horses).

ELISA tests are the most sensitive screening assays available. Different ELISA protocols, targeting antibodies against different epitopes and with different test procedures were described for WNV diagnosis (Murgue et al., 2001; Durand et al., 2002; Autorino et al., 2002; Jourdain et al., 2009; Angelini et al., 2010). Different ELISA tests are used in animals.

IgM-capture ELISA (MAC-ELISA), used in chickens and wild birds, it is optimal for detecting recent infections, cross reactions with antibodies against other viruses are less frequent than by using IgG ELISA (Weingartl et al., 2003; Johnson et al., 2003).

IgG-ELISA was standardized for human sera and adapted for horses and several bird species (Ebel et al., 2002; Weingartl et al., 2003; Dauphin and Zientara, 2007). In horses, IgG lasts for at least 15 months after infection. A positive result on a single sample has limited clinical diagnostic value and it is necessary to demonstrate a recent infection by a four-fold increase in antibody titre on paired serum samples collected 14 days apart (Castillo-Olivares and Wood, 2004).

Epitope-blocking ELISAs utilize various MAb and are species-independent (Blitvich et al., 2003a and 2003b; Sotelo et al., 2011).

In general, cross-reactions with antibodies against other flaviviruses (St Louis encephalitis, dengue, yellow fever) are possible when using ELISA tests. High cross-reactivity rate is found among viruses belonging to the same antigenic complex, like Usutu virus, a virus in the same WNV serogroup (Japanese Encephalitis complex). Therefore, ELISA positive samples should be confirmed by serum neutralization test.

IHA are labor-intensive and non specific due to cross reaction with related flaviviruses.

Complement fixation test is more laborious than ELISA and it is not routinely used for WNV antibodies detection. CFT test was historically used for serosurveys (particularly until the 1950's) and are considered more specific than most other serological tests, although serological cross-reactions with other flaviviruses may still occur. Complement fixing antibodies appear later in infection than haemagglutination inhibition antibodies, but have a shorter half life (around two years); therefore this test is sometimes used in attempts to determine the time of infection.

Seroneutralization tests allow discriminating between infections by different flavivirus and are considered the "gold standard" for WNV antibody detection. On the other hand, they are time consuming, expensive and require live virus. As a consequence, seroneutralization tests are not suitable for large scale testing. Different protocols are describeb, based on differences of the virus strains used, the dimension of plate (micro virus-neutralization test) and the endpoint titers of cytopatic effect (PRNT 50 or PRNT 90) (Tsai et al., 1998; Savage et al., 1999; Lundström, 1999; Weissenböck et al., 2003; Buckley et al., 2003; Rizzoli et al., 2007; Linke et al., 2007; Jiménez-Clavero et al., 2008; Hubálek et al., 2008; Lopez et al., 2008; Jourdain et al., 2008a; Macini et al., 2008; Jourdain et al., 2009; Monaco et al., 2009; Balança et al., 2009; Calistri et al., 2010; Angelini et al., 2010).

IFA is a valuable confirmatory assay for avian species. This procedure is very sensitive but also time consuming and laborious. It is not useful for mammalian serum due to non specific binding of IgM MAb (Kauffman et al., 2003).

Information on animal response to the infection (i.e. occurrence of clinical signs in horses, antibody response, localization of WNV in bird organs) have been integrated into a descriptive model, together with key aspects of WNV transmission cycle (see Annex D for details). The objective of such a comprehensive approach is to favour interpretation of available data, and to guide proposal for data collection.

4.1.3. Data collection on WNV in animals by competent authorities of EU countries, Norway and Switzerland.

Summary of results of questionnaires to the Task Force on zoonoses data collection

Twelve MSs (Denmark, Estonia, Finland, France, Hungary, Italy, Latvia, Portugal, Romania, Slovakia, Slovenia, and Sweden) and Norway and Switzerland responded to the questionnaires regarding WNV.

WNV is notifiable in animals in Denmark, Finland, France, Italy, Latvia, Portugal, Romania, Slovenia, and Sweden.

In France, Italy, Portugal, Romania, Switzerland and Sweden active and passive data collection are carried out. In Denmark, Finland, Hungary and Slovenia only passive surveillance activity is carried out.

In Estonia, Latvia, Slovakia and Norway no data collection activity (active/passive) in animals is carried out.

Country by country results

Country by country results are reported in Annex A. Main information is summarised in Table 19.

Table 19. Summary of results of the questionnaire on data collection on WNV in animals: active and passive data collection[a]

Country	Active data collection			Passive surveillance			National Reference Laboratory
	Time span	*Animal species*	*Diagnosis*	*Time span*	*Animal species*	*Diagnosis*	
Denmark							National Veterinary Institute Technical University of Denmark
Finland				2010 – present	Wild birds	RT-PCR	Finnish Food Safety Authority
France	2000 – 2007	*Anseriformes*, equids	ELISA	2001 – present	Wild birds, equids	RT-PCR	Maisons-Alfort laboratory for studies and research on animal disease and zoonoses
Hungary				2003 – present	Wild birds	IHC, RT-PCR	Central Agricultural Office Veterinary Diagnostic Directorate
				2009 – present	Equids	Indirect ELISA, RT-PCR	
Italy	2008 – present	*Corvidae*	RT-PCR	1998 – present	Equids		Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"
	2001 – present	Other *Passeriformes*	RT-PCR			Indirect ELISA	
	2001 – present	Chickens in rural farms, equids, cattle	Indirect ELISA				
Portugal	2010 – present	Equids, wild birds		2006 – present	Equids, wild birds		Laboratório Nacional de Investigação Veterinária. Direcção Geral de Veterinária
Romania	2009 – present	*Anseriformes*, chickens, equids	ELISA	2009 – present	Chickens, equids		National reference laboratory for arboviroses: bluetongue and african horse sickness
		Ciconiformes, *Falconiformes*	Virus isolation				
Slovenia					Equids	ELISA	National Veterinary Institute
Sweden	2006	Equids	ELISA	2009 – present	Wild birds, wild animals	IHC, RT-PCR	National Veterinary Institute
	2005 – 2006	Migratory birds	ELISA				
Switzerland				2005 – present	Wild birds	RT-PCR	University of Zurich

(a) No data collection activities nor reference laboratory were reported in the questionnaires from Latvia, Norway and Estonia. IHC: immunohystochemistry

4.1.4. Systematic literature review of data collection on WNV in animals

The literature search was carried out on publications from 1998 through 2010. Publications were retrieved from 14 countries out of 29 considered in the search. Results by country, including information on diagnostic method and sampling design, as well as specific bibliographic references are presented in Annex B. Based on the systematic literature review, results of active and passive data collection are separately analysed and reported.

Results of active data collection on WNV by animal species

Given the epidemic pattern of WNV in Europe, and given the different role of animal species in the transmission cycle, we separately report results on indirect and direct diagnostic tests, in birds and in other animal species. In fact, indirect tests reveal past exposure to the virus, whereas direct tests usually indicate current infections and may more easily be associated with recent outbreaks of transmission even in those species that rarely undergo clinical and pathological consequences.

Results of active data collection in birds

A summary of results of the systematic literature review on active data collection on WNV in birds, by indirect diagnostic tests, is reported in Table 20. Fifteen publications from eleven countries (Austria, Czech Republic, France, Germany, Greece, Italy, Poland, Romania, Spain, Sweden, and United Kingdom) were examined. Prevalence of antibodies against WNV in birds, belonging to several orders, which were tested in the greatest number of countries, was very variable. Relatively low prevalence levels were found in *Passeriformes*, which were most frequently tested. *Columbiformes* were found positive with the highest prevalence that was however mostly due to a single study in Italy (Monaco et al., 2009). *Galliformes* might include sentinel chickens in selected high-risk areas. Potential cross reactions with other Flavivirus must be taken into account in the interpretation of results of indirect tests.

Results of active data collection on WNV in birds by direct tests, from eight publications in four countries (Czech Republic, France, Italy, Sweden) are summarised in Table 21. Prevalence of WNV by direct test was low in *Passeriformes*, whereas greater prevalence in *Charadriformes* can be explained by sampling of birds following outbreaks in high risk areas.

Table 20. Results of active data collection WNV birds in the EU, Norway and Switzerland, by using indirect diagnostic tests, based on the systematic review of publications from 1998 through 2010[(a)].

Group	N of countries	N of studies	N of tested animals	N of positive animals	Prevalence	Lower 95% CL	Upper 95% CL
Passeriformes[(b)]	9	139	8690	284	3.27	1.88	5.62
Anseriformes[(c)]	7	12	496	20	4.03	1.66	9.49
Galliformes[(d)]	6	13	375	101	26.93	17.41	39.20
Corvidae [(e)]	5	16	672	78	11.61	4.25	27.97
Ciconiiformes[(f)]	5	9	1063	32	3.01	1.49	6.00
Columbiformes[(g)]	5	6	271	97	35.79	18.72	57.44
Charadriiformes[(h)]	4	7	584	3	0.51	0.21	1.26
Falconiformes[(i)]	4	6	53	0	0.00	0.00	0.07
Birds[(j)]	4	4	47	3	6.38	1.15	28.63
Coraciiformes[(k)]	3	4	7	1	14.29	2.76	49.44
Accipitriformes[(l)]	2	9	264	31	11.74	4.52	27.23
Strigiformes[(m)]	2	7	36	2	5.56	0.72	32.30
Gruiformes[(n)]	2	3	183	22	12.02	7.14	19.53
Gaviformes[(o)]	2	2	46	10	21.74	5.36	57.66
Phoenicopteriformes[(p)]	1	2	269	37	13.75	8.48	21.54
Piciformes[(q)]	1	2	5	0	0.00	0.00	0.52
Pelecaniformes[(r)]	1	1	15	5	33.33	14.60	59.40
Cuculiformes[(s)]	1	1	2	0	0.00	0.00	0.84

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study. (b) Balança et al., 2009, Buckley et al., 2003, Jourdain et al., 2009, Jourdain et al., 2007b, Jourdain et al., 2008a, Linke et al., 2007, Lopez et al., 2008, Lundström, 1999, Savage et al., 1999; (c) Buckley et al., 2003, Figuerola et al., 2007, Hubálek et al., 2008, Jourdain et al., 2009, Linke et al., 2007, Lundström, 1999, Savage et al., 1999; (d) Buckley et al., 2003, Hubálek et al., 2008, Lopez et al., 2008, Lundström, 1999, Rizzoli et al., 2007, Savage et al., 1999, Tsai et al., 1998; (e) Balança et al., 2009, Buckley et al., 2003, Hubálek et al., 2008, Jourdain et al., 2008b, Linke et al., 2007, Lundström, 1999; (f) Balança et al., 2009, Figuerola et al., 2007, Hubálek et al., 2008, Jourdain et al., 2008a, Linke et al., 2007, Lundström, 1999; (g) Hubálek et al., 2008, Lopez et al., 2008, Lundström, 1999, Monaco et al., 2009; (h) Balança et al., 2009, Figuerola et al., 2007, Hubálek et al., 2008, Jourdain et al., 2009; (i) Balança et al., 2009, Buckley et al., 2003, Jourdain et al., 2009, Linke et al., 2007; (j) Jourdain et al., 2009, Jourdain et al., 2008a, Monaco et al., 2009, Tsai et al., 1998; (k) Jourdain et al., 2008a, Linke et al., 2007, Lopez et al., 2008; (l) Buckley et al., 2003, Linke et al., 2007; (m) Balança et al., 2009, Linke et al., 2007; (n) Linke et al., 2007; (o) Lundström, 1999; (p) Figuerola et al., 2007; (q) Linke et al., 2007; (r) Lundström, 1999; (s) Linke et al., 2007.

Table 21. Results of active data collection WNV birds in the EU, Norway and Switzerland, by using direct diagnostic tests, based on the systematic review of publications from 1998 through 2010[(a)].

Group	N of countries	N of studies	N tested	N positives	Prevalence	Lower 95% CL	Upper 95% CL
Charadriiformes[(b)]	3	5	24	6	25.00	6.79	60.39
Passeriformes[(c)]	3	4	230	7	3.04	0.76	11.41
Corvidae[(d)]	2	10	2035	86	4.23	2.68	6.61
Columbiformes[(e)]	2	5	504	39	7.74	4.27	13.62
Birds[(f)]	1	4	557	15	2.69	1.25	5.70
Pelecaniformes[(g)]	1	1	7	1	14.29	1.97	58.06

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study. (b) Angelini et al., 2010, Calistri et al., 2010, Jourdain et al., 2009, Lundström, 1999, Calistri et al., 2010, Lundström, 1999, Monaco et al., 2009, Savini et al., 2008; (c) Angelini et al., 2010, Jourdain et al., 2009, Jourdain et al., 2007b, Calistri et al., 2010; (d) Angelini et al., 2010, Calistri et al., 2010, Jourdain et al., 2008b, Monaco et al., 2009, Savini et al., 2008; (e) Lundström, 1999, Monaco et al., 2009, Savini et al., 2008; (f) Angelini et al., 2010, Calistri et al., 2010, Monaco et al., 2009; (g) Calistri et al., 2010.

Positive results in birds, by direct diagnostic tests, were found in Italy (150 birds), Czech Republic (three birds), and France (three birds).

Results of active data collection in equids

Indirect tests were used in active data collection in horses in Italy (six studies), Austria (two studies), France (one study) and in Poland (one study) (Table 22). Overall prevalence of positive tests, as estimated by taking into account clustering at the country level, was 15.2% (3.3, 48.1). This result is affected by relatively high prevalence in Italian studies. Major variability across European studies is accounted for by the wide confidence interval of prevalence estimate.

Table 22. Prevalence and 95% confidence interval in active data collection on WNV in horses, by indirect test, based on the systematic review of publications from 1998 through 2010[(a)].

Country	N of studies	N of tested horses	N of positive horses	Prevalence	Lower 95% CL	Upper 95% CL
Italy[(b)]	5	1242	432	34.78	31.8	37.89
France[(c)]	1	5107	182	3.56	3.09	4.11
Austria[(d)]	2	385	4	1.04	0.03	2.78
Poland[(e)]	1	78	0	0.0	0.0	4.6

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study; (b) Autorino et al., 2002, Monaco et al., 2009, Angelini et al., 2010; (c) Durand et al., 2002; (d) Weissenböck et al., 2003; (e) Hubálek et al., 2008.

Direct diagnostic tests were used in horses in two studies in Italy, where 25 horses out of 800 tested were positives (3.1%, exact binomial 95% CI: 2.0, 4.6) (Savini et al., 2008, Monaco et al., 2009).

Results of active data collection in cattle

Active data collection on WNV in cattle was carried out in Italy by indirect tests (Calistri et al., 2010). Prevalence of positive cattle was 2.3% (1.6, 3.2).

Results of passive data collection on WNE by animal species

Results of passive data collection, by country and species (birds and equine) are shown in Table 23. Confirmed cases of WNV in birds include data from an outbreak in domestic goose and in a rehabilitation centre, in Hungary. Greatest numbers of confirmed cases in horses were reported in Italy and France

Table 23. Summary of results of passive data collection on WNV by country and animal species, based on the systematic review of publications from 1998 through 2010.

Country	Species or group	Number of data collection activities	Number of suspected cases	Number of confirmed cases
Austria	Birds[(a)]	1	97	0
Austria	*Equus caballus*[(b)]	1	14	0
France	*Equus asinus*[(c)]	1	2	1
France	*Equus caballus*[(d)]	2	201	89
Hungary	*Accipiter gentilis*[(e)]	4	6	6
Hungary	*Accipiter nisus*[(f)]	1	1	1
Hungary	*Anser anser*[(g)]	3	59	58
Italy	*Equus caballus*[(h)]	5	143	117
Netherlands	*Equus caballus*[(i)]	1	71	0
Spain	*Aquila adalberti*[(j)]	1	21	5
Spain	*Aquila chrysaetos*[(k)]	1	2	2
Spain	*Hieraaetus fasciatus*[(l)]	1	1	1
United Kingdom	Birds[(m)]	2	2030	0

(a) Weissenböck et al., 2003; (b) Weissenböck et al., 2003; (c) Murgue et al., 2001; (d) Leblond et al., 2007, Murgue et al., 2001; (e) Bakonyi et al., 2006, Erdélyi et al., 2007; (f) Erdélyi et al., 2007; (g) Bakonyi et al., 2006, Glávits et al., 2005; (h) Angelini et al., 2010, Cantile et al., 2000, Macini et al., 2008, Monaco et al., 2009; (i) Rockx et al., 2006; (j) Höfle et al., 2008; (k) Jiménez-Clavero et al., 2008; (l) Jiménez-Clavero et al., 2008; (m) Phipps et al., 2008.

4.1.5. Information on the occurrence and geographical distribution of West Nile virus, hosts and vectors

Agent

Countries where West Nile virus is reported in animals are shown in Figure 7, based upon systematic literature review and from the epidemiological bulletin of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy.

Positive countries are those where animals (of any species) were found positive for WNV, regardless of the type of used test (direct or indirect) and of the type of data collection (active or passive). The Netherlands were classified as negative since suspect cases of WNV were reported in horses by passive data collection, but were not subsequently confirmed (see also Table23).

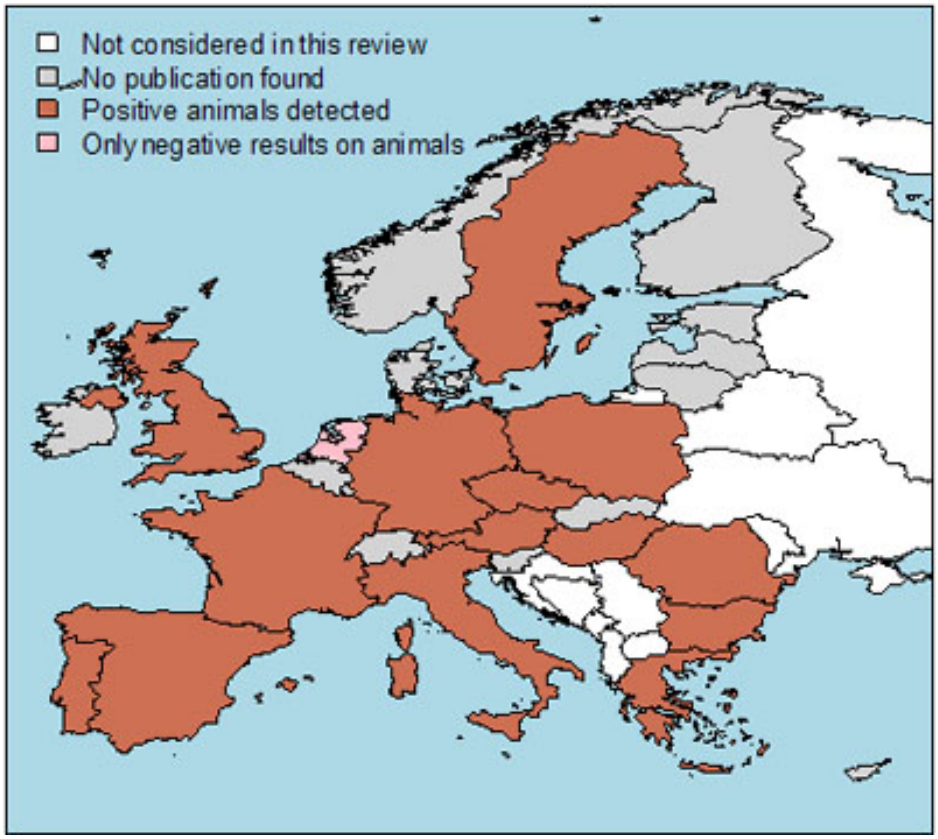

Figure 7. Map of the distribution of countries where WNV in animals was reported, either by active or passive data collection, from 1998 through January 2011[11].

Vectors

Distribution of vectors of West Nile virus in European countries is shown in Table 24 (data obtained by Snow and Ramsdale, 2011; Schaffner et al, 2001; Snow and Ramsdale, 1999).

[11] Austria: Lundström et al., 1999, Weissenböck et al., 2003; Bulgaria: Epidemiological bulletin of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", 2010; Czech Republic: Lundström et al., 1999; France: Balanca et al., 2009, Durand et al., 2002, Jourdain et al., 2007b, Jourdain et al., 2008a, Jourdain et al., 2008b, Leblond et al., 2007, Murgue et al., 2001; Germany: Linke et al., 2007; Greece: Lundström et al., 1999; Hungary: Bakonyi et al., 2006, Erdélyi et al., 2007; Erdélyi et al., 2007; Bakonyi et al., 2006, Glávits et al., 2005; Italy: Angelini et al., 2010, Autorino et al., 2002, Calistri et al., 2010, Cantile et al., 2000, Lundstrom et al., 1999, Macini et al., 2008, Monaco et al., 2009, Rizzoli et al., 2007, Savini et al., 2008; Netherlands: Rockx et al., 2006; Poland: Hubálek et al., 2008, Lundström et al., 1999; Portugal: Barros et al., 2011; Romania: Lundstrom et al., 1999, Savage et al., 1999, Tsai et al., 1998; Spain: Figuerola et al., 2007, Höfle et al., 2008, Jiménez-Clavero et al., 2008, Lopez et al., 2008; Sweden: Jourdain et al., 2009, United Kingdom: Buckley et al., 2003, Phipps et al., 2008.

Table 24. Distribution of important WNV-vector mosquito species in UE countries, Norway, and Switzerland.

	Austria	Belgium	Bulgaria	Cyprus	Czech Republic	Denmark	Estonia	Finland	France	Germany	Greece	Hungary	Ireland	Italy	Latvia	Lithuania	Luxembourg	Malta	Netherlands	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	United Kingdom	Switzerland	Norway
Culex pipiens	x[(a)]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Culex mosdestus	x		x		x				x	x	x	x		x						x	x	x	x	x	x		?		
Culex perexiguus			x	?							x			x							x				x				

(a), x= present; ? = Doubtful.

Note: data were obtained from Snow and Ramsdale, 2011; Schaffner et al, 2001; Snow and Ramsdale, 1999.

Hosts
The distribution of the main hosts of WNV in Europe can be found in Annex F.

4.2. Proposal for data collection on WNV in animals

Based on the results obtained from the data inventory and on the application of criteria (described in Annex C), it was decided to consider as candidates to be involved in data collection: equids (active and passive data collection), birds belonging to the order *Passeriformes* (active and passive data collection), all birds species (passive date collection).

Other mammal species could be used as sentinel in a monitoring scheme for WNV, mainly when they are already sampled and tested for other pathogens. In Italy and Spain, bovids were considered as alternative mammal sentinels (involved in official monitoring program and in research studies). The bovid samples conserved in serum banks may be used for retrospective survey on WNV, but *ad hoc* research should be carried out to verify the usefulness of this type of data collection. No clinical manifestation was reported in Europe.

Proposals for data collection are described below and summarised in Table 25 and Table 26.

4.2.1. Data collection on WNV in equids.

Equids are the object of many research studies and official monitoring program in several MS. The relatively high intensity of mosquito exposure makes them more likely to be infected than people. Horses are routinely visited and monitored by veterinarians. Equines are routinely bled and tested for other pathogens, such as Equine infectious anaemia.

The use of equine WNV vaccine may decrease the incidence of WNV disease, but it also results in positive serological tests. For this reason, the vaccinated horses must be excluded from serological surveys.

Horses can show neurological signs that can be easily detected by veterinarians. WNV infection in equines ranges from asymptomatic to fatal encephalitis. Approximately the 10% of horses infected with WNV presents neurological disorders. The outcome of WNV infection probably depends on host factors and virus strain (Castillo-Olivares and Wood, 2004).

Specification on active data collection on WNV in equids.
Subpopulations to be covered
Equids that were not vaccinated against WNV should be included

Agents and possible subtypes
No distinction among WNV lineages should be made in data collection.
Sampling context and design, sampling stage
Equids can be sampled at owner (for companion animals) of at the farm level (for farm animals). The ideal time for sampling is from July to September; this period may be extended from May to October, particularly in the Mediterranean countries. In countries or administrative divisions where blood serum from equids is collected for tests on equine infectious anemia, a random sample of those sera may be tested for WNV. In other areas, a random sample of animals should be selected from a list. For farm animals, a multistage sampling of herd and, subsequently, individuals, should be carried out (see Annex E on sampling for details).

A stratified design, at the level of second order administrative divisions, should be carried out, if possible. Stratification at the first order administrative division can be used otherwise. When population size of each stratification area is available, the number of sampled animals might be proportionate. If for example, a area accounts for 10% of equids of a country, 10% of the total sample size for the country should be collected from this area. In any case, information on the horse

population in each administrative area would be useful, and it could be used in the application of weights in case of disproportionate stratified sampling (see Annex E for details on sampling).

For sample size for the detection of trends, please refer to Annex E on sample size.

For the detection of the presence of WNV in an animal population, the following indications could be considered. Testing 100 animals would allow the detection of the infection, with 95% confidence, if WNV prevalence is 3.6%. Such a prevalence was reported in France by indirect diagnostic tests of horses; furthermore it is lower than the prevalence of 4.9% and 4.2% that were found in *Corvidae* in Europe by indirect and direct diagnostic test, respectively. It is to be noted that these calculations assumes perfect sensitivity and specificity of laboratory tests and random selection of animals, therefore, these factors must be considered when drawing conclusions on the presence of WNV in a sampled population.
For animals in farms, sample size must be accordingly increased due to non independence of observations (see section on sample size determination).
Type of specimen to be taken
Blood specimens should be taken for the serological detection of antibodies against WNV.
Diagnostic methods to be used
It is recommended to use an indirect ELISA test based on the detection of IgM in order to detect an acute infection. Positive cases should be confirmed by a confirmatory test (PRNT, seroneutralisation test). By this approach, the focus is on the detection of recent infections.
Data submission
Details on stage of sampling (individual animals, or farm animals) should be reported.

Specification on passive data collection on WNV in equids
Subpopulations to be covered
All equids presenting clinical signs that can be caused by infection by WNV can be considered as suspect cases (see below).
Agents and possible subtypes
No distinction among WNV lineages should be made in data collection.
Sampling context and design, sampling stage
Suspect cases should be reported, by private veterinarians, to competent authorities and laboratory confirmation should be carried out. Suspect cases are defined based upon the following clinical signs:

- weakness (hind end, front end, or both, characterized by falling to their knees or difficulty in remaining standing),
- ataxia (incoordination), muscle twitching or tremors;
- altered mental state (somnolent or aggressive, circling, or stall-walking);
- hypersensitivity to touch or sound;
- cataplexy or narcolepsy;
- seizures;
- blindness;
- cranial nerve deficits (facial paralysis, tongue weakness, difficulty swallowing);
- recumbency;
- fever.

In the differential diagnosis the following pathogens, causing diseases with similar neurological signs, have to be considered:

- Eastern equine encephalitis (EEE) – not present in Europe
- Western equine encephalitis (WEE)– not present in Europe

- Japanese encephalitis (JE) – not present in Europe
- Neural sarcocystosis (caused by *Sarcocystis neurona*)
- Borna disease
- Equine Herpesvirus 1
- Rabies
- Bacterial meningitis

Type of specimen to be taken and diagnostic tests to be used
For indirect diagnosis, ELISA for the detection of IgM in serum can be used in order to detect an acute infection. Positive cases should be confirmed by a confirmatory, seroneutralisation test (PRNT).

For direct diagnosis, immunocytochemistry on central nervous system (including cortex, cerebellum, brain stem and spinal cord), and RT-PCR on blood are recommended (virus isolation would provide further confirmation of the infection). It is however to be noted that equine tissues generally contain lower concentrations of virus than birds and viremia duration is very short.

Data submission
Details on type of diagnosis for confirmation (direct, or indirect tests) should be reported.

Type of specimen to be taken and diagnostic tests to be used
For indirect diagnosis, ELISA for the detection of IgM in blood serum can be used in order to detect an acute infection. Positive cases should be confirmed by a confirmatory, seroneutralisation test (PRNT).

For direct diagnosis immunocytochemistry on central nervous system (including cortex, cerebellum, brain stem and spinal cord), and RT-PCR on blood are recommended (virus isolation would provide further confirmation). It is however to be noted that equine tissues generally contain lower concentrations of virus than birds and viremia duration is very short.

Data submission
Details on type of diagnosis for confirmation (direct or indirect tests) should be reported.

4.2.2. Data collection on WNV in birds

Specfications for active data collection on WNV in birds
Although wild birds, such as *Passeriformes*, are less available for sampling in comparison with domestic species, these animals serve as major hosts for the mosquitoes and are reservoirs for WNV and, therefore, are most useful as indicators of WNV transmission in a geographic area. *Passeriformes* are involved in official monitoring programmes for WNV and other diseases in several Member States and in research studies (see results from questionnaires and systematic literature review). On the other hand, migrations prevent determination of areas where exposure of birds to WNV occurred.

Subpopulations to be covered
Synanthropic bird species—those that live within or in proximity to human settings, such as *Corvidae* (crows, jays, magpie), but also other species such as starlings, are of particular interest in data collection, both for their potential role as sources of infection of humans, and for the relative ease of capture, that is in some cases carried out for population control plans. Bird species other than *Passeriformes*, such as *Culumbiformes*, showed high prevalence in active data collection and could be selected for the monitoring. Within these species, a choice can be made, country by country, based on ease of capture and pre-existing capture programs, including data collection program on WNV or other agents.

Sampling context and design, sampling stage

Sampling can be carried out within capture activities, for population control or other purposes, or during *ad-hoc* capture for WNV testing. Stratification at the level of second level administrative division would improve precision of prevalence estimates and would allow the analysis of trends at a relatively small scale.

Type of specimen and sampling technique
The most suitable samples include and pool of organs (kidney, spleen, brain, heart), whole blood, blood serum. Blood and pools of organs can be sampled from euthanized or shot animals within specific wildlife population control programmes. Bird tissues generally contain higher concentrations of virus than equine tissues. Viraemia in *Passeriformes* experimentally infected with WNV only lasts one week, and the probability of finding a viraemic bird in nature is minute, therefore, blood samples are of limited usefulness (Komar et al., 2003).

Diagnostic methods to be used

It is recommended a direct diagnosis (RT-PCR) on pool of organs sampled from birds, in order to detect viral acid nucleic in tissues. Even if the sampling is labor intensive, tests are highly sensitive, specific, rapid but expensive. Confirmation by viral isolation would be useful.

Indirect diagnosis by ELISA tests can be used on blood serum, but positive results must be confirmed due to the possibility of cross reactions with other Flavivirus.

Data submission
Details on the type of diagnostic test used should be provided to favour interpretation of results.

Specifications for passive data collection on WNV in birds
In Europe, birds do not usually show clinical signs following infection by WNV. Nevertheless, WNV should be considered as a potential cause in any case of abnormal mortality in wild birds. This is justified by the possibility of importation of a new strain of the virus, pathogenic to birds, or unexpected viral mutations with consequences on patogenicity for birds.

Subpopulations to be covered

Wild birds are the most favourable target.

Agents and possible subtypes

No distinction among WNV lineages should be made in data collection.

Sampling context and design, sampling stage
Suspect cases should be reported by veterinarians and by personnel involved in wildlife management and monitoring (including wildlife protection institututions, hunting associations). The above mentioned personnel should inform health authorities (i.e. veterinarians, reference laboratories) of the finding of suspect cases during their ordinary outdoor activities. Data collection by personnel going outdoor specifically to look for suspect cases is not included in this proposal.

Suspect cases are defined based upon increased, unexplained mortality, or clinical signs involving the nervous system, such as generalized weakness, inability to stand, tremor, paralysis of the limbs, drooping on the wings, circling (based on cases of disease observed in North America).

Subsequent confirmation should be based on laboratory diagnosis.

Type of specimens and diagnostic tests

Pool of organs, including central nervous system, and whole blood should be collected. RT-PCR is recommended (virus isolation would provide further confirmation).

Data submission

Details on type of diagnosis for confirmation (direct or indirect tests) should be reported.

Table 25. Summary of proposals for active data collection on WNV in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimens	Diagnosis
Equids	Animals not vaccinated against WNV	Owner, at farm, from July to September, or May-October in Southern Europe	Convenience or random sampling based on a list, multistage sampling for farm animals. Stratified by administrative area	Blood serum	IgM-ELISA, confirmatory seroneutralization
Birds	Synanthropic *Passeriformes Corvidae*: crows, jays, magpies, and other species, starlings Other bird species	*Ad hoc* captures, population control capture or killing	Stratified by administrative area	Pool of organs (kidney, spleen, brain, heart), whole blood, serum	RT-PCR, ELISA and confirmatory seroneutralization

Table 26. Summary of proposals for passive data collection on WNV in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimens	Diagnosis
Equids	All animals showing clinical signs involving the CNS	Suspect cases reported by owner and veterinarians		Blood serum, cortex, cerebellum, brain stem and spinal cord	IgM-ELISA, confirmatory seroneutralization, immunocytochemistry, RT-PCR
Birds	Birds affected by mortality or clinical signs involving the CNS	Suspect cases reported by veterinarians, wildlife management personnel		Pool of organs, whole blood, serum	RT-PCR, virus isolation

4.3. References on WNV

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5. TICK-BORNE ENCEPHALITIS VIRUS

Tick-borne encephalitis (TBEV) virus belongs to the genus *Flavivirus*, family *Flaviviridae* (Grard et al., 2007). It is the most important arboviral infection of the human central nervous system in Europe and Russia, which can result in death or long-term sequelae (Donoso Mantke et al., 2008).

TBEV is endemic in an area stretching from north of China and Japan, across Russia into Europe (Mansfield et al., 2009). According to serological and genetic characteristics and geographical distribution, three subtypes of TBE virus are known: European, Far-Eastern and Siberian. The three TBEV subtypes are associated with various degrees of disease severity. The geographical distribution of the viral subtypes coincides with the distribution of vectors (Süss, 2003).

TBE is a seasonal disease, depending on the activity of ticks, and is often biphasic in central Europe (April to July and September to October) and monophasic in northern Europe (June to September) and in the Mediterranean area (November to January) (Leshnik et al, 2002).

Humans are accidental hosts for TBEV, and can get infected by tick-bite or by the ingestion of raw unpasteurized milk and dairy products from viremic animals (Dumpis et al., 1999). In Europe, there are about 2800 TBE confirmed cases per year (Süss et al., 2008) and an increasing incidence in all European endemic countries is being observed. In Austria an intensive vaccination campaign is carried out (Arnez and Avsic-Zupanc, 2009; Heinz, 2008).

5.1. Inventory of available data

5.1.1. Transmission cycle of TBEV, role of animal hosts

TBEV is maintained in cycles involving *Ixodid ticks* and vertebrates. Ticks act both as virus vectors and natural reservoir hosts. Vertebrate hosts amplify the virus by acting as a source of infection for feeding ticks (Labuda et al., 2002).

Virus transmission takes place horizontally between vectors and vertebrates (Donoso Mantke et al., 2008). After feeding on a viremic host, ticks remain infected for life (Süss, 2003). The virus proliferates in the tick, enters the cells of the midgut wall, and the infection of salivary glands and saliva allows viral transmission during the following blood meal (Nuttall et al., 1994). Trans-ovarial transmission also occurs, and it is important in maintaining long-term viral circulation in natural foci (Danielova et al., 2002). Co-feeding of infected and non-infected ticks on the same host plays a major role in virus transmission among ticks without the need of viraemia in the host (Labuda et al., 1996; Randolph et al., 1996).

Ixodes ricinus is the main vector of the European subtype of TBEV, while the Far-Eastern and Siberian subtypes are transmitted by *Ixodes persulcatus* (Lindquist and Vapalahti, 2008). Where these tick species are absent, other species of the genera *Ixodes*, *Haemaphysalis* and *Dermacentor* can serve as vectors of the virus (Süss, 2003).

In contrast to other tick-transmitted diseases, TBE is distributed in an endemic pattern of natural discrete foci over a wide geographic area in central Europe, the Baltic States and the Russian Federation, and it is absent in many regions where both ticks and hosts abound (Süss, 2003). Endemic foci are characterised by a high rate of autumnal cooling that promotes a specific pattern of ticks' seasonal dynamics, namely seasonal feeding-synchrony of larvae and nymphs, which facilitates virus transmission through co-feeding on rodents (Labuda et al., 1993, 1997; Randolph et al., 1996, 1999, 2000; Labuda and Randolph, 1999).

Small mammals are excellent hosts for immature ticks and are important for maintenance of the cycles and as reservoirs (Süss, 2003). Indeed, they are suitable natural hosts of the virus due to short generation time and high reproductive rate; furthermore, they are important in the process of co-feeding, since they can be hosts for several ticks at the same time. Both rodents and insectivores have

a sufficient viraemia to allow the ticks to become infected while feeding. The main TBEV hosts in Europe are the bank vole (*Myodes glareolus*), yellow-necked mouse (*Apodemus flavicollis*), squirrel (*Sciurus vulgaris*) and the hedgehog (*Erinaceus europaeus*) (Avsic-Zupanc and Petrovec, 1996; Dobler et al., 2010). Some wild carnivores (mustelids and subadult foxes) are also competent reservoirs (Süss, 2003).

The persistence of TBEV foci also requires the presence of large hosts, which support tick populations being the main hosts for adult ticks. Some even-toed ungulates (*Capreolus, Cervus, Rupicapra, Sus, Alces, Bos, Ovis, Capra*), birds (passerine and game birds), lagomorphs, bats, and some carnivores (adult foxes, dogs), feed ticks but are incompetent as reservoirs for TBEV (Süss, 2003).

Domestic dogs can develop systemic infection and can potentially infect feeding ticks but, from the point of view of the transmission in nature, can be considered as dead-end hosts; the same conclusions can be extended to wild canids, such as foxes and wolves, and to horses.

Domestic ruminants (goats, sheep, cattle) can excrete the virus through milk during their viraemia and determine food-borne outbreaks (Dumpis et al., 1999). However, they have a limited role in infecting ticks. Experimental studies in ruminants showed no virus presence in skin excisions taken from the sites of infected feeding tick; it is therefore unlikely that co-feeding transmission occurs like in small mammals. Even lactating animals that can transmit the virus through milk to the offspring or to humans do not develop sufficient viraemia for transmission of the virus to the tick (Labuda et al., 2002).

A diagram was drawn in order to visualize the transmission cycle of TBEV and the role of animals (Figure 8). Here, arrows represent transmission of the virus, whereas animals are divided into four main categories: reservoir hosts, acquiring the infection from infected ticks, and passing it to susceptible ticks (as shown by the two arrows); incompetent hosts, that may be exposed to TBEV, but do not, generally, infect susceptible ticks (only one, unidirectional, arrow); domestic ruminants, that may be exposed to TBEV, do not generally infect susceptible ticks (unidirectional arrow) but may transmit the virus through milk (dashed arrow); dogs, that might infect ticks (dashed arrow), but with no role in transmission. Man may acquire the infection from ticks (black arrow) and by consumption of raw unpasteurized milk from domestic ruminants (dashed arrow) and develop clinical disease. This schematic representation will be used as a starting point for the selection of animal species for data collection (see section 5.2).

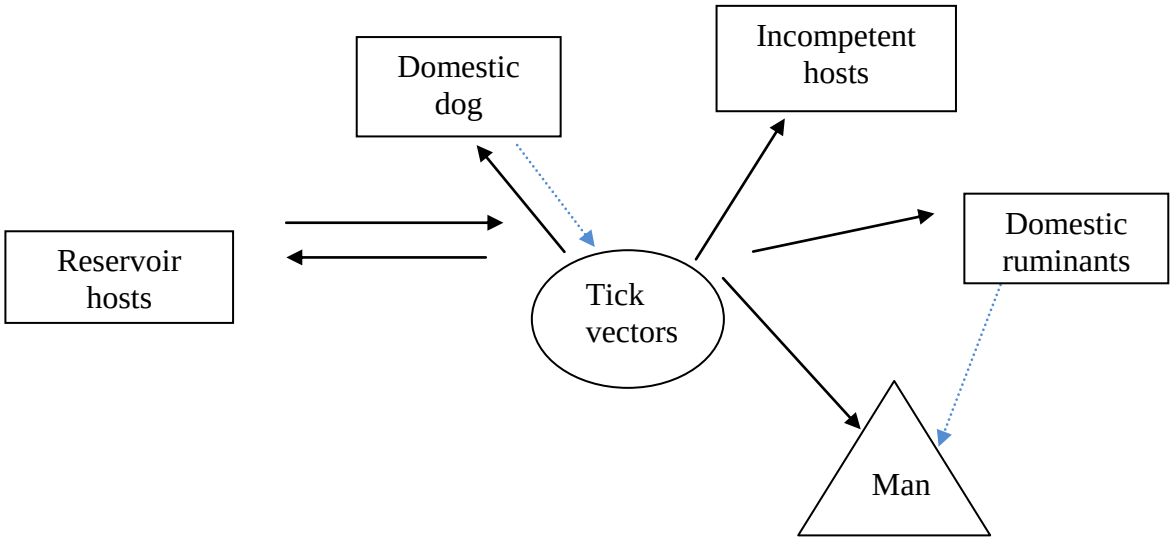

Figure 8. Diagram of the transmission of TBEV with specific focus on the role of animals.

5.1.2. Diagnosis of TBEV in animals.

Clinical signs in animals caused by TBEV

In animals, clinical cases of TBE are infrequent. TBE with neurological symptoms (cerebrocortical, thalamic, and brainstem) have been described in dogs for more than 30 years (Leschnik et al., 2002). Rarely, the disease has been observed in horses (Waldvogel et al., 1981; Grabner, 1993) and monkeys (Süss et al., 2007 and 2008; Klaus et al., 2010a).

Laboratory diagnosis of TBEV in animals

TBEV infection has uncharacteristic clinical features, and laboratory diagnosis is required, using either direct or indirect methods.

Direct laboratory diagnosis

Virus isolation is performed in tissue cultures or by inoculation in suckling mice. It can be carried out in viremic animals, likewise the reverse transcription polymerase chain reaction (RT-PCR). During viraemia, the virus is present in blood, cerebrospinal fluid (CSF) and tissues of infected animals (Leschnik et al., 2002; Holzmann, 2003). However, viraemia is short and does not exceed 10 days in most species (Heinz, 2008; Balogh et al., 2010).

Certain target organs (brain, kidney, spleen, salivary glands and brown fat) are known to contain virus for several weeks (10-12) after its disappearance from the blood (Perez-Eid et al., 1992).

Other direct diagnostic methods include the viral antigens detection by immunohistology and immunihistochemistry in brain tissues and cytological tests of blood and CSF (Leschnik et al., 2002).

Direct diagnostic methods such as virus isolation and histological exams are generally used in passive surveillance on dead animals. RT-PCR is mostly used in research studies, in a lesser extent routine diagnostics.

Indirect laboratory diagnosis

Serological tests for antibody detection are the most used tests to detect TBEV infection in animals, although the number of evaluated methods for veterinary use is small (Klaus et al., 2010a). Available indirect tests include ELISA, sero-neutralization test (NT), hemagglutination inhibition (HI) and indirect immunofluorescence assay (IFA). Tests are performed on sera, and to a lower extent, on CSF and milk.

The ELISA test for antibodies detection (IgM and IgG) is the test of election. ELISA wells can be coated either with inactivated TBEV or purified TBEV antigens. IgM detection allows an early diagnosis of primary infection with TBEV, while IgG in animals can persist for several months (Klaus et al., 2010a and b; Donoso Mantke et al, 2008; Lindhe et al., 2009; Csango et al., 2004; Roelandt et al., 2009; Wurm et al., 2000; Sikutová et al., 2009; Balogh et al., 2010; Stadtbäumer et al., 2004).

An 'all-species' test for IgG detection in animals is commercially available and is recommended for antibody examination, but for research use only (Klaus et al., 2010b). Moreover, a commercial blocking ELISA is available for animals without the need of anti-IgM/IgG animal-specific coniugates (Venturi et al., 2009). However, in most cases, animals are tested with modified indirect ELISAs intended for human diagnostics, which are used in combination with species-specific IgM/IgG conjugates.

Sensitivity of TBEV ELISA tests is reportedly high (98-100%), while specificity is low due to the high cross-reactivity among all *Flaviviridae* (production of non-neutralizing antibodies yielding a positive result in the case of yellow fever, Japanese encephalitis, dengue, WNV antibodies). For this reason, the use of ELISA as the sole serologic diagnostic method when testing animals may be insufficient, and ELISA results need to be confirmed by NT.

The specific immunity against TBEV can only be assessed in NT assays (Balogh et al., 2010; Lindhe et al., 2009). NT is used as single test in animals (Kocianová et al., 1993; Kozuch et al, 1990 and 1995; Rizzoli et al., 2007) and frequently as confirmatory test. NT is considered the gold-standard test, but it is laborious and time-consuming, as cell cultures and live virus are needed.

HI test is also used in animals, as a single test or in series, to be confirmed by ELISA/NT (Balogh et al., 2010; Hubálek et al., 1986; Juceviciene et al., 2005; Klimes et al., 2001; Traavik et al., 1984; Kahl and Radda, 1988; Gîrjabu et al., 1985).

IFA, commonly used for routine human diagnostics, was reported as highly aspecific when used to test goat samples (Balogh et al; 2010).

5.1.3. Data collection on TBEV in animals by competent authorities of EU countries, Norway and Switzerland.

Summary of results from questionnaires

Ten MSs (Denmark, Estonia, Finland, France, Hungary, Latvia, Portugal, Romania, Slovakia, Sweden) and Norway and Switzerland responded to the questionnaires regarding TBE. This disease is notifiable in animals in Latvia and Romania only.

Past or current data collection on TBEV in animals was reported in France, Latvia and Sweden. Active data collection is carried out in Sweden, passive data collection in France and Latvia.

Country by country results

Country by country results are reported in Annex A, main information is summarized in Table 27.

Table 27. Summary of results of the questionnaire on data collection on TBEV in animals: active and passive data collection.

Country	Active data collection			Passive surveillance			National Reference Laboratory
	Time span	*Animal species*	*Diagnosis*	*Time span*	*Animal species*	*Diagnosis*	
Denmark							National Veterinary Institute Technical University of Denmark
France					deer		*Centre National de Référence des Arbovirus, Institut Pasteur*
Hungary							The Central Agricultural Office
Latvia				1999-present	Not specified	Indirect ELISA	Institute of Food Safety, Animal Health and Environment 'BIOR'
Romania							NRL for Arboviroses: bluetongue and African horse sickness
Sweden		insectivores	RT-PCR				

[a] No data collection activities nor reference laboratory were reported in the questionnaires from Estonia, Finland, Norway, Portugal, Slovakia, Switzerland.

5.1.4. Systematic literature review on data collection on TBEV in animals.

The literature search was carried out without limiting the time span. Indeed, a low number of publications about TBEV detection in animals was retrieved when literature search was limited to the past ten years only. Thirty-seven publications, from 16 countries, were found relevant for our data search on active data collection on animals. The publications on passive data collection were three, from one country.

Results by country are presented in Annex B.

Results of active data collection by animal species

Main results of active data collection are summarized in Table 28. Small mammals (including rodents and squirrels), dogs, domestic ruminants (goats, sheep, cattle) and wild ruminants were the object of the greatest numbers of data collection activities. Data on domestic ruminants, small mammals and dogs were collected from the greatest numbers of countries.

Table 28. Results of active data collection on TBEV in animal species in the EU, Norway and Switzerland, based on the systematic review of publications (no time limits)[(a)].

Animal species	N of countries	N of studies	N of tested animals	N of positive animals	Prevalence (%)	Lower 95% CL	Upper 95% CL
Small mammals[(b)]	7	11	5837	720	12.34	7.59	19.43
Dog[(c)]	7	8	2641	362	13.71	7.40	23.99
Cattle[(d)]	7	7	1780	128	7.19	2.67	17.98
Goat[(e)]	6	8	2827	218	7.71	3.39	16.58
Sheep[(f)]	6	6	1710	95	5.56	3.63	8.42
Bird[(g)]	4	4	857	14	1.63	0.25	9.87
Deer[(h)]	3	7	427	57	13.35	10.92	16.22
Pig[(i)]	3	3	439	19	4.33	0.84	19.49
Horse[(j)]	3	3	386	13	3.37	1.54	7.23
Fox[(k)]	2	3	1259	50	3.97	0.47	26.77
Hare[(l)]	1	2	449	21	4.68	3.19	6.80
Mouflon[(m)]	1	1	80	6	7.50	3.41	15.70

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study; (b) Kocianová et al., 1993, Kozuch et al., 1990, Kozuch et al., 1995, Molnár et al., 1976, Rizzoli et al., 2004, Traavik et al., 1984, Weidmann et al., 2006; (c) Chambouris et al., 1989, Comin et al., 2007, Csango et al., 2004, Kirtz et al., 2003, Klimes et al., 2001, Lindhe et al., 2009, Reiner et al., 2002, Ruatti et al., 1985; (d) Comin et al., 2007, Daneš et al., 1992, Gîrjabu et al., 1985, Hubálek et al., 1986, Juceviciene et al., 2005, Koptopoulos and Papadopoulos, 1980, Sikutová et al., 2009; (e) Balogh et al., 2010, Gîrjabu et al., 1985, Hubálek et al., 1986, Juceviciene et al., 2005, Koptopoulos and Papadopoulos, 1980, Nekrošienė et al., 2003, Rizzoli et al., 2007, Stimbirys et al., 2006; (f) Comin et al., 2007, Gîrjabu et al., 1985, Hubálek et al., 1986, Juceviciene et al., 2005, Koptopoulos and Papadopoulos, 1980, Sikutová et al., 2009; (g) Gîrjabu et al., 1985, Juricová et al., 1998, Juricová and Hubálek, 1999, Koptopoulos and Papadopoulos, 1980; (h) Comin et al., 2007, Daneš et al., 1992, Juricová and Hubálek, 1999; (i) Gîrjabu et al., 1985, Juricová and Hubálek, 1999, Koptopoulos and Papadopoulos, 1980; (j) Comin et al., 2007, Koptopoulos and Papadopoulos, 1980, Sikutová et al., 2009; (k) Rieger et al., 1999, Wurm et al., 2000; (l) Daneš et al., 1992, Juricová and Hubálek, 1999; (m) Juricová and Hubálek, 1999.

Results of passive data collection on TBEV in animals

Passive data collection on TBEV in animals is summarised in Table 29. Publications are from Austria and correspond to case reports in dogs. The presence of infection was confirmed by immunohistology and immunihistochemistry in brain tissues in two collection activities, while serology was employed to detect TBEV antibodies in one activity. Although TBEV infection has aspecific clinical sign, most suspect cases were confirmed by laboratory tests.

Table 29. Passive data collection TBEV in animals based on the systematic review of publications.

Animal species	Country	Criteria per suspected case definition	N of suspected cases	N of confirmed cases	Diagnostic test	Tissue type
Dog[(a)]	Austria	acute vision loss, neurological signs	1	1	indirect serology (IgG)	serum, CSF
Dog[(b)]	Austria	fever, myoclonus, convulsions, hemiplegia, tetraparesis, stupor, anisocoria	4	4	histochemistry	Purkinje cells, neurons of brain stem nuclei, neuronal cell processes and cytoplasm of macrophages
Dog[(c)]	Austria	neurological signs	8	5	histology, immunohistology	brain tissues

(a) Stadtbäumer et al. (2004); (b) Weissenböck and Holzmann (1996); (c) Weissenböck et al. (1998).

5.1.5. Information on the occurrence and geographical distribution of TBEV, hosts and vectors

Agent

TBEV is distributed in an area ranging from north of China and Japan, across Russia into Europe, including areas of Central Europe and Scandinavia (Mansfield et al., 2009). Almost all known isolates from Europe belong to the European subtype, while the Siberian subtype is found in the Urals, Siberia and fareastern Russia, and the Far Eastern subtype in far-eastern Russia, China and Japan (Donoso Mantke et al., 2008).

In Europe, TBEV is present in natural foci in the geographic range of the vectors, *I. ricinus* and *I. persulcatus*. However, unlike *B. burgdorferi* sl, which can be considered as widespread across the entire range of *I. ricinus* (although with variable prevalence in ticks and animal hosts), foci of TBEV are limited to certain areas. The reasons behind the heterogeneous geographic distribution of TBEV are still to be clarified. According to current hypotheses, TBEV would be likely to be established at those locations where, due to climatic conditions, immature stages of *I. ricinus* (larvae and nymphs) are both active during the same season (i.e. spring). Under these circumstances, TBEV would be able to be transmitted to larvae feeding on animal hosts that were recently infected by nymphs. On the contrary, transmission would not occur at the many locations where larvae mostly feed in summer, well beyond the duration of infectivity of animal hosts that usually infected nymphs in spring (Randolph, 1999).

In the systematic literature review on data collection in animals, positive results were reported from all countries where data collection activities were carried out: Austria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Norway, Poland, Romania, Slovakia, Switzerland. These countries are classified as positive in Figure 9. Details for each country, including information on diagnosis, are reported in Annex B.

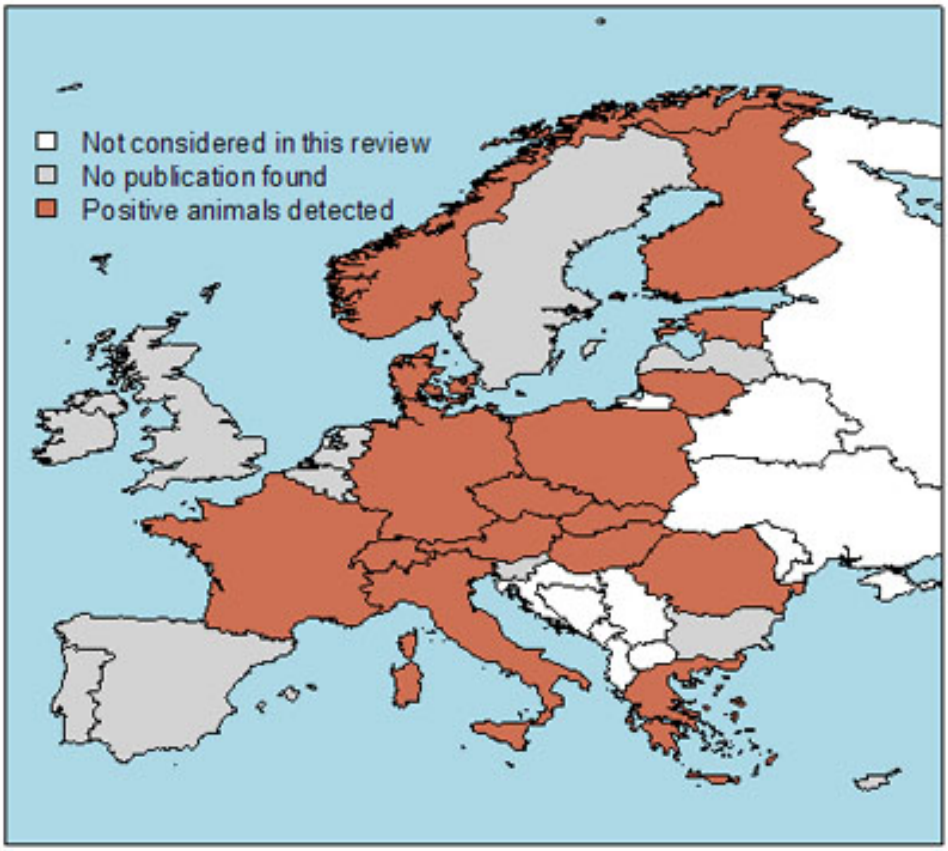

Figure 9. Distribution of TBEV in animals, by active data collection, based on systematic literature review. Positive countries are those where animals positive to TBEV were found[12].

Hosts

The presence of the most relevant sylvatic host species (small mammals) for TBEV in EU MSs, Norway and Switzerland is illustrated in Annex F. In the same section, data on distribution of the main large mammals serving as hosts for TBEV tick vectors (roe deer, red deer, Eurasian elk, and fallow deer) and estimates of some domestic animals which can get infected by TBEV (cattle, goat, sheep and horse) are presented.

Vectors

The geographical distribution of *I. ricinus* is illustrated in Figure 2 in *B. burgdorferi* sl section, section 1.1.5.

5.2. Proposal for data collection on TBEV in animals

To select animal species to be included in data collection, we built an initial list of species involved in the transmission cycle of TBEV based on the narrative and systematic literature reviews. A set of criteria was subsequently applied to select species involved in options for data collection. Details on the application of criteria can be found in Annex C. General conclusions that were drawn in this process are listed below.

[12] Austria: Kirtz et al., 2003, Kozuch et al., 1995, Stadtbäumer et al. 2004; Weissenböck and Holzmann, 1996; Weissenböck et al., 1998; Czech Republic: Daneš et al., 1992, Juricová and Hubálek, 1999, Klimes et al., 2001, Weidmann et al., 2006; Denmark: Lindhe et al., 2009; Estonia, Golovljova et al., 2004; Finland: Brummer-Korvenkontio et al., 1973; France: Rieger et al., 1999; Germany: Kahl et al., 1988, Kocianová et al., 1993, Rieger et al., 1999, Wurm et al., 2000; Greece: Chambouris et al., 1989, Koptopoulos and Papadopoulos, 1980; Hungary: Balogh et al., 2010, Molnár et al., 1976, Sikutová et al., 2009; Italy: Comin et al., 2007, Rizzoli et al., 2004, Rizzoli et al., 2007, Ruatti et al., 1985; Lithuania: Juceviciene et al., 2005, Nekrošienė et al., 2003, Stimbirys et al., 2006; Norway: Csango et al., 2004, Traavik et al., 1984; Poland: Juricová et al., 1998; Romania: Gîrjabu et al., 1985; Slovakia: Hubálek et al., 1986, Kozuch et al., 1990; Switzerland: Reiner et al., 2002.

Based on the application of the criteria mentioned above, we decided to consider as candidates to be involved in data collection: dogs (for active and passive data collection), domestic ruminants (active data collection), and wildlife competent reservoir hosts (active data collection).

Wild ungulates (deer, wild bovids and wild boar) serve as host for the tick vectors, are exposed to the virus, and the prevalence of antibodies in TBEV foci can reach relatively high levels, especially in deer (Juricová and Hubálek, 1999). The red fox is considered a good indicator animal for natural TBEV foci, due to its high levels of infestation by ticks and high mobility in the environment (Rieger et al., 1999); fox sera collected as part of the rabies control programs have been used also for TBEV investigation (Wurm et al., 2000).

However, these wild species are less available for harmonised sampling in comparison with domestic species. The sampling of wild animals can be logistically complicated, more time-consuming and costly as compared to domestic animal species.

Horses are susceptible to TBEV and may occasionally suffer clinical signs. Harmonised sampling from horses is possible; however their geographic distribution and frequentation of habitat favourable to tick vectors might be very heterogeneous across Europe.

A summary of proposals of data collection on TBEV in animals is presented in Table 30.

5.2.1. Data collection on TBEV in dog

Dogs are frequently used in research studies and as indicators of the risk of TBEV. Advantages of dog over other species include widespread distribution and close association with humans, with which dogs may share exposure to outdoor environments and ticks. Furthermore, dogs are more likely to be exposed to tick bites in comparison with people frequenting the same habitats.

Specification on active data collection on TBEV in dogs

Subpopulations to be covered

Privately-owned dogs and shelter dogs may be included in data collections. Information can be collected, conditioned upon owners' availability and compliance, on exposure to ticks, provenience, exposure to wooded habitat (favourable to *I. ricinus* and TBEV), and treatments that might interfere with antibody response. The obvious limit of the use of shelter dogs is the lack of the above information. On the other hand, shelter dogs are easily accessed for sampling.

Sampling should only include resident dogs or dogs that do not travel outside the country of residence regularly frequenting outdoor habitats, below 1000 m above sea level. Dogs that are regularly treated with repellents, including collars and spot-on repellents, should be excluded or, alternatively, such information should be reported

Sampling context and design, sampling stage

Privately owned dogs should be sampled in the context of visits by veterinarians. Shelter dogs can be more easily accessed by competent authorities. Given difficulties in the realization of a formal random sampling of owned dogs, convenience sampling is to be adopted, based on availability by owners and veterinarian, and in compliance with inclusion criteria described above.

Serum samples are easily collected from dogs that can also be routinely sampled and tested for other agents (including vector-borne agents). Information on exposure to outdoor habitat and movements should be gathered by interviewing owners.

In each country, sampling design should be stratified at least at the level of first order administrative areas, or at the level of second order administrative areas, if possible. This would increase the probability of collecting a representative sample of the entire country, without the need for the

application of weights to adjust for disproportionate stratified sampling (EFSA 2009) (see Annex E for further details).

Type of specimen to be taken and sampling technique

For active data collection on dogs, the collection of sera is recommended, for the subsequent search of antibodies against TBEV.

Diagnostic methods to be used

Serology is considered the method of choice for diagnosis in dogs. A high percentage of infected dogs show an adequate immune response by developing high antibody titre. The antibodies to TBEV are detectable in serum samples for more than 9 months and in CSF for more than 2 months (Leshnik et al., 2002). No specific commercial test is available for dogs, but all-species tests can be used or tests for humans which are adapted by using anti-dog IgM/IgG conjugates. However, the use of ELISA as the sole serologic diagnostic method may be insufficient due to the low specificity, and the specific immunity against TBE virus must be assessed by NT (Lindhe et al., 2009).

Data submission

Data collected, preferably separate by first order administrative level to account for within country spatial variations, should include the number of tested and of positive individuals. Privately owned and shelter dogs (if considered) should be clearly distinguished. The effects of geographic location and of dog type should in this way be considered at the level of trend analysis, to obtain comparable estimates of trends.

Specification on passive data collection on TBEV in dogs

The usefulness of passive surveillance of TBEV in dogs for the study of trends of the agent is reduced by the uncharacteristic clinical picture and the lack of defined clinical diagnostic criteria. In spite of these problems, we propose to record the number of suspect cases and the number of confirmed cases, as indexes of the presence of the agent in a geographic area. Obviously, the interpretation of these results will be cautious.

Subpopulations to be covered

All dogs presenting clinical signs that can be caused by infection by TBEV can be considered as suspect cases (see below). Those individuals that never frequented outdoor environment and, therefore, contact with tick vectors is very unlikely. Imported and dogs travelling among different countries should be excluded from sampling.

Sampling context and design, sampling stage

In the case of passive data collection, suspect cases should be reported by private veterinarians, to competent authorities and laboratory confirmation should be carried out.

Definition of suspect cases should be based on most common signs of the infection. Dogs infected by TBEV can show a peracute, acute and chronic course of infection. Neurological symptoms are rare and half of the seropositive dogs do not develop clinical signs (Weissenböck et al., 1998). However, fever and neurological signs such as myoclonus, convulsions, hemiplegia, tetraparesis, stupor, acute vision loss and anisocoria have been reported (Stadtbäumer et al., 2004; Weissenböck et al., 1998; Weissenböck and Holzmann, 1996). In the early stages of the disease, leukopenia and lymphopenia is a common finding, whereas in chronic stages of TBE or after administration of steroids leukocytosis is eminent (Leshnik et al, 2002).

Type of specimen to be taken and diagnostic tests to be used

The demonstration of specific serum antibodies by serological tests (NT, or ELISA and NT in series) is the method of choice for the confirmation of suspect TBE in live animals. Laboratory confirmation

of suspect cases can be also performed by RT-PCR on blood, cerebrospinal fluid and tissues, in the firs phases of infection: as viraemia is temporary, the detection of the viral nucleic acid is only possible during a short period in the early stage of infection (Leshnik et al., 2002). Cytological tests of blood and cerebrospinal fluid can be performed too.

In dead dogs, immunohistological demonstration of TBEV antigen in brain tissues is a useful method for proving the diagnosis (Weissenböck et al., 1998).

Differential diagnoses to TBE are distemper, rabies, toxoplasmosis, neosporosis, pseudorabies, borreliosis, bacterial meningitis, and granulomatous meningoencephalitis (Leshnik et al., 2002).

Data submission

Reporting should include both the number of suspect cases, and the number of confirmed cases, as well as the size of the canine population, at the level of first administrative areas.

5.2.2. Data collection on TBEV in domestic ruminants.

Among domestic animals, ruminants may play a significant role in TBE risk by maintaining tick populations, thereby increasing human exposure; also, unpasteurized raw milk (especially goats and sheep, more rarely cattle) is known to be a possible source for human disease (Klaus et al., 2010b). A well demonstrated response antibody response is present in goats, sheep and cattle. In goats, TBEV enters the circulation on the 4th day after infection. Neutralizing antibodies first appear on days 10–13 after transmission (Nosek et al., 1967) and can persist for more than one year (Klaus et al., 2010a). The virus is secreted with milk in low concentrations during the viremic phase for 3-7 days, beginning as early as the second or third day post infection (Van Tongeren, 1955; Gresikova, 1958a; Gresikova 1958b; Gresikova and Rehacek, 1959).

There seems to be a general agreement between seropositivity among domestic animals, ticks and human cases of TBE (Juceviciene et al., 2005). In particular, goats and sheep were the object of several studies on TBEV, indeed they are usually left to graze in ecotonal areas that represent a suitable habitat for the questing activity of *I. ricinus* (Rizzoli et al., 2007). Moreover, diagnosis can be made both in blood and milk, whose collection is easy. Only active data collection is proposed for domestic ruminants due to the absence of TBE clinical signs.

Specification on active data collection on TBEV in domestic ruminants

Subpopulations to be covered

Animals with frequent access to outdoor environment, below 1000 m asl should be considered, such as free-ranging herds. In fact, although *I. ricinus* is currently expanding its range above such an altitude in certain countries, establishing an altitudinal limit for sampling would improve harmonisation of data collection.

Sampling context and design, sampling stage

Sampling may occur at the farm level. A multistage sampling can be implemented, by randomly selecting herds from a list, and then testing serum from randomly selected individuals within each herd. As a consequence, clustering of observations needs to be taken into account when determining sample size. After the selection of herds, sera can be tested for antibodies against TBEV (see below) among those that were collected for other disease control programs (i.e. brucellosis, Infectious Bovine Rhinotracheitis, etc.).

In the case of milk testing, herds can be randomly selected and a sample from the farm milk can be analyzed to detect TBEV infection in the entire herd.

Stratification at the first order administrative level, or, if possible, at the second order administrative level is recommended.

Type of specimen and sampling technique

Blood serum and milk for subsequent antibody detection tests.

Diagnostic methods

During viraemia the virus is detectable by PCR in blood and milk of infected animals. However, as the viremic period is short (in goats, until days 8–10 after infection depending on the amount of virus inoculated), detection of antibodies by serologic methods is considered a better diagnostic tool (Klaus et al., 2010b).

Antibodies can be detected in serum and milk of domestic ruminants. No specific commercial ELISA tests are available for domestic ruminants, but all-species tests can be used or tests for humans which are adapted by using anti-bovine or small ruminants IgM/IgG conjugates. Indeed, when testing goat sera, a human adapted to goat IgM ELISA showed better performances when compared to the commercially available 'all-species' test for IgG detection (Klaus et al., 2010b). Like in dogs, sensitivity of TBEV ELISA tests is high, while specificity is low due to the high cross-reactivity among *Flaviviridae* and the specific immunity against TBE virus must be confirmed by a NT assay.

Data submission

Number of tested and positive animals or, in case of analyses at herd level (test performed on farm milk), the number of tested and positive herds should be reported. Data relative to animals raised in herd should be accompanied with information on the number of tested herds and herd where at least one positive animal was found. Moreover, herd size (number of animals per herd) should be reported, for the proper interpretation of results based on varying within-herd sample size, and as a factor associated with husbandry practices that might affect exposure to TBEV.

5.2.3. Data collection on TBEV in small rodents.

Wildlife competent reservoir species have been studied in several epidemiological surveys and could serve as infection sentinels for TBEV. They are relatively easy to capture, show a persistent TBEV infection, and are ubiquitous in all ecological systems (Achazi et al., 2011). In Europe, the main TBEV hosts are bank vole (*Myodes glareolus*), yellow-necked mouse (*Apodemus flavicollis*), squirrel (*Sciurus vulgaris*) and the hedgehog (*Erinaceus europaeus*) (Avsic-Zupanc and Petrovec, 2006).

The proportion of small rodents with antibodies against TBEV, together with the average number of larvae and nymphs feeding on each small mammals host, has been shown to be an indicator of the variation of human morbidity with a 1-2 year lag in Russia (Moshkin et al., 2009). In Germany, a recent study showed that TBEV detection in rodents represents a good marker system for TBE, particularly in areas of low TBEV circulation (Achazi et al., 2011).

Specifications on active data collection on TBEV in small rodents

Subpopulations to be covered

Rodents that live in areas that are frequented by humans should be covered. Therefore, data collection should include wild rodents in natural areas frequented mostly for recreational or occupational reasons.

Sampling context and design, sampling stage

In active data collection on wild rodents, land areas should be the primary sampling units. Accordingly, each country could be divided into 20 by 20 km squares. One square should then be randomly selected in each first order administrative area, retaining only those including wooded areas frequented by people. If squares with such characteristics are not selected, other squares should be selected, in each areas, until a suitable one is found. Availability of personnel in protected natural areas (i.e., natural parks) could also be a factor in the inclusion of locations for data collection.

Rodents should be trapped overnight by using live traps, during monthly sessions in spring and summer. It is suggested that sampling is carried out across an entire country, and not only previously known TBEV foci. In fact, this approach would provide more valid information on the geographic distribution of TBEV and would allow the detection of previously unknown infected areas.

Type of specimen to be taken and sampling technique

Captured rodents should be anesthetized for the collection of blood and then euthanized for the collection of organs (to be preserved frozen). For trapping and handling rodents, national legislations must be considered, as well as rules for personnel protection (http://www.cdc.gov/rodents/).

Diagnostic methods to be used

In rodents, RT-PCR on tissues can be considered the test of election to detect TBEV. The viraemia is usually short, lasting from 2 to 8 days (Heinz, 2008; Perez-Eid et al., 1992; Ernek et al., 1963; Heigl and Von Zeipel, 1966), but, once infected with TBEV, small mammals are supposed to develop a persistent infection (Ernek et al., 1963; Achazi et al., 2011). Target organs are brain, kidney, spleen, salivary glands and brown fat (Perez-Eid et al., 1992).

Serological tests (hemagglutination inhibition, serum neutralization) can also be used to detect TBEV antibodies in small mammals (Moshkin et al., 2009; Perez-Eid et al., 1992). However, the variability of individual animals is such that only a small percentage retains virus and develops antibodies (Perez-Eid et al., 1992). Moreover, antibodies, which can first be detected at about the 13th day after infection, disappear by the end of the second month. TBEV can persist in the host as a latent infection and experimental studies in small mammals showed a reactivation by pharmacological immunodepressants and stress hormones (Moshkin et al., 2009).

Data submission

Geographic coordinates of trapping locations should be provided. Data on the number and species of trapped animals, and number and species of positive animals, should be submitted in order to analyze TBEV infection in the different geographical areas.

Table 30. Summary of proposed active data collection activities on TBEV in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimens	Diagnosis
Dog	Animals frequenting outdoor habitat < 1000 m altitude	Owner, by private veterinarians	Convenience or random sampling based on lists. Multistage sampling for farm animals. Stratified by administrative area	Blood serum, and milk for ruminants	Indirect ELISA confirmed by NT test
Domestic ruminants		At farm			
Wild rodents	Animals frequenting wooden areas < 1000 m altitude	Live trapping and euthanasia, in 20 by 20 km land units.	Stratified by administrative area	Tissues samples	RT-PCR

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6. HANTAVIRUS

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are a group of antigenically distinct, negative-strand RNA viruses (Plyusnin et al., 1996). In humans, some Hantaviruses tend to be associated with asymptomatic infections or mild disease, whereas others have high case fatality rates. Hantaviruses are causes of two main diseases in humans: Hemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS). Hantavirus infections were probably heavily under-diagnosed in the past; a major reason for this is that better diagnostic tests have only recently become available (Vaheri et al., 2008).

6.1. Inventory of available data on Hantavirus in animals

6.1.1. Transmission cycle of Hantavirus

Unlike other viruses belonging to the family *Bunyaviridae*, Hantaviruses are not transmitted by arthropod vectors, but rodent reservoir hosts are considered as responsible for their maintenance in nature and for transmission to humans and other animals. Insectivores were also recently identified as potential reservoir hosts (Song et al., 2007).

Rodents excrete Hantavirus in urine, feces and saliva for weeks or even their entire life. The virus may survive several days in the environment, depending on physical and chemical factors (Vaheri et al., 2008). Infection of man and other animals may take place by direct contact with rodent excrements, or by inhaling infected dust by aerosol. Transmission may also occur through broken skin, conjunctiva and other mucous membranes, by rodent bites and, possibly, by ingestion of contaminated food and water.

Animal species other than rodents (cat, dog, fox, deer, hare, cattle, pigs) produce detectable antibodies against Hantavirus (Malecki et al., 1998; Leighton et al., 2001; Ahlm et al., 2000; Escutenaire et al., 2000b; Khaiboullina et al., 2005). In agreement with Zeier et al. (2005), a potential role of these species in the transmission of Hantavirus to man cannot be ruled out with certainty, and further studies on the subject are needed. However, in summarising the transmission cycle of Hantavirus (as shown in the diagram in Figure 10) we adhere to the most commonly shared view that only rodents pose a risk for humans and are the ecological pillar for viral perpetuation. Other animal species are, therefore, considered as dead-end hosts. Accordingly, arrows in Figure 10 represent transmission only ocurring from reservoir hosts or, most commonly, from contaminated environment.

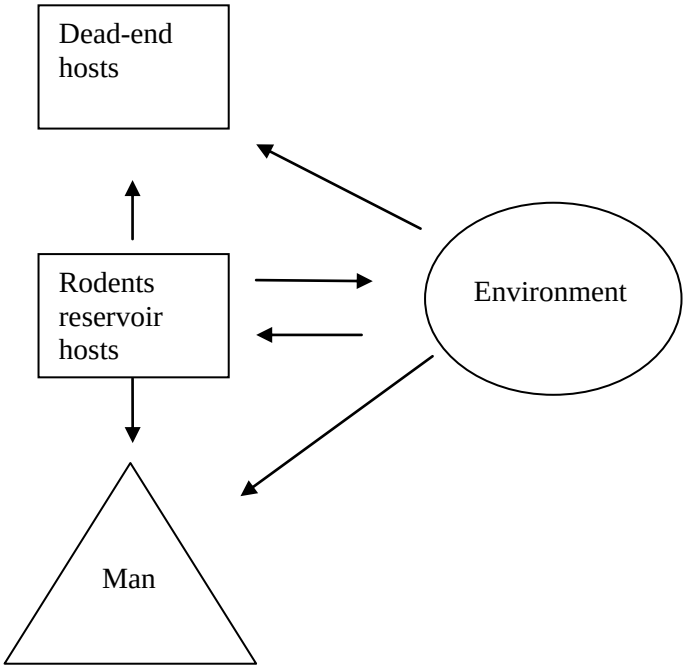

Figure 10. Diagram of the transmission of Hantavirus with focus on the role of animals.

Each Hantavirus subtype is associated with a single rodent (or insectivore) species. Rodent reservoir hosts belong to four genera: *Myodes*, *Microtus*, *Apodemus*, *Rattus* (Plyusnin and Morzunov, 2001; Kallio et al., 2006). Moreover, clinical consequences of human infection vary across subtypes. The association between Hantavirus subtype, rodent reservoir hosts, and human disease is summarised below.

Puumala (PUUV), carried by the bank vole (*Myodes glareolus*), causes HFRS and HPS in man.

Tula (TULV), carried by the common vole (*Microtus arvalis*); the role of TULV as agent of human disease is uncertain.

Dobrava, (DOBV), carried by the yellow-necked mouse (*Apodemus flavicollis*), causes HFRS.

Saaremaa (SAAV), carried by the striped mouse (*Apodemus agrarius*), causes mild form of HFRS.

Hantaan (HNTV), carried by *A. agrarius*, causes HFRS.

Seoul (SEOV), carried by brown and black rats (*Rattus norvegicus*, *R.rattus*), causes HFRS.

The bank vole, *M. glareolus* (the carrier of PUUV) is the most important European reservoir host of Hantavirus (Brummer-Korvenkontio et al., 1980; Jonsson et al., 2010). *Apodemus* spp. is associated with DOBV, which was isolated from *A. flavicollis* captured in Dobrava village, Slovenia (Avsic-Zupanc et al., 1992). Later, a new DOBV-like virus was isolated from *A. agrarius* captured in Tula region in European Russia (Plyusnin et al., 1999). Another DOBV-like isolate was recovered from *A. agrarius* captured on the Saaremaa island of Estonia (Nemirov et al., 1999). This new genetic lineage was also accepted as a new virus species, named Saaremaa virus (SAAV) (Sjölander et al., 2002), after it was found in a natural reservoir in different European countries (Heyman et al., 2009b).

Microtus spp. carries TULV, but currently no clinical disease has been clearly associated with any *Microtus*-associated Hantavirus (Scharninghausen et al., 2002).

SEOV is harboured by *R. norvegicus* (Lee et al., 1980). Its worldwide distribution is a result of rat global distribution. In Europe a SEOV persistence was demonstrated in a population of *R. norvegicus* captured in France, but no human infection in Europe was reported so far (Heyman et al., 2004).

The presence of Hantavirus antigens in rodent species different from the elective reservoir was also documented. For example, TULV antigens were found in the yellow-necked mouse (*A. flavicollis*), wood mouse (*A. sylvaticus*), bank vole (*M. glareolus*), and European pine vole (*Microtus subterraneus*) (Heroldová et al., 2010). PUUV infection found *M. arvalis* and *A. sylvaticus* (Zeier et al., 2005).

No information is available on the pathogenicity of insectivore-borne Hantavirus.

6.1.2. Diagnosis of Hantavirus in animals.

Laboratory diagnosis of Hantavirus in animals

Direct laboratory diagnosis

RT-PCR is the direct test most widely used in direct diagnosis of Hantavirus in animals, especially in sacrified rodents. Lungs were shown as the organs where virus detection is most likely, although heart, kidneys, or blood serum can also be tested, but with a relatively low sensitivity (Essbauer et al., 2006). RT-PCR is targeted to a fragment of the S and/or M RNA segment encoding for a nucleocapsid protein (N). Depending on used primers, RT-PCR can be able to detect all rodent- associated Hantavirus, or one specific Hantavirus subtype (Avsic-Zupanc et al., 2007). Subtype characterisation can be also achieved after sequencing of RT-PCR products. Direct IFA is also used. Direct laboratory diagnosis can be employed to confirm previous positive results using indirect tests (see below).

Indirect laboratory diagnosis

ELISA, IFA and WB are most commonly used in research. Commercial kits available for humans are often used on animals, after making some changes in the protocols and/or in the reagents (Aberle et al., 1999; Heyman et al., 2002a; Heyman et al., 2002b; Heyman et al., 2009b).

Subtype-specific serological tests are available, but cross reaction between serotypes occurs. Therefore, although in the diagnosis of Hantavirus infection, sensitivity and specificity of antibody detection by ELISA and IFA may be greater than 97%, other tests are necessary to identify the infecting subtype (Reusken et al., 2008; Vaheri et al., 2008; Dobly et al., 2011).

Focus reduction neutralization test (FRNT) is the only indirect test that can be used for confirmation and serotyping of Hantavirus infection in animals, and it is considered as a gold standard (Klingström et al., 2002; Vaheri et al., 2008). However, FRNT is difficult to perform and it is carried out in few laboratories. As a consequence, in many studies, Hantavirus serotypes are not identified, but conclusions are tentatively drawn based on the rodent host species and previous findings in the same area, if available (Dobly et al., 2011).

6.1.3. Data collection on Hantavirus in animals by competent authorities of EU countries, Norway, Switzerland.

Summary of results of questionnaires to the Task Force on zoonoses data collection

Twelve countries responded to the questionnaire on Hantavirus: Denmark, Estonia, Finland, France, Hungary, Latvia, Portugal, Romania, Slovakia, Sweden, Norway, Switzerland.

Hantavirus is not notifiable in any of these countries.

Active data collection in animals was carried out in Sweden and in Switzerland.

Country by country results of questionnaires

In Sweden, the University of Umea is responsible for active data collection on Hantavirus in rodents in selected areas. ELISA is used for indirect diagnosis.

In Switzerland, Spiez Laboratory is responsible of the active data collection on rodents in selected areas in the country. A prevalence study on PUUV, TULV, and SAAV has been carried out, in 2010, on *Myodes glareolus* and *Microtus arvalis*, by using RT-PCR.

6.1.4. Systematic literature review of data collection on Hantavirus in animals

The literature search was carried out on publications from 2000 through 2010, and from 1990 for those countries where no publication was found in the 10-year time span. Results by country and information on diagnostic tests and sampling are presented in Annex B.

Results of active data collection on Hantavirus in animals

Main results of active data collection are summarized in Table 31. For the calculation of prevalence, data from 51 publications were used, from 19 countries: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Portugal, Slovakia, Slovenia, Sweden, Switzerland, United Kingdom. One publication was found from Norway, but data on the number of tested animals were not available. Small rodents were the objects of the greatest numbers of data collection activities. Data on small rodents were collected from greatest numbers of countries. Only two studies collected data on non rodent animals.

Table 31. Results of active data collection on Hantavirus in animal species in the EU, Norway and Switzerland, based on the systematic literature review of publications from 2000 to 2010[(a)].

Animal species	N countries	N of data collections	N of tested animals	N of positive animals	Prevalence (%)	Lower limit (%)	Upper limit (%)
Myodes glareolus[(b)]	14	40	12004	2107	17.55	12.89	23.44
Apodemus flavicollis[(c)]	12	18	3224	117	3.63	0.88	13.76
Microtus arvalis[(d)]	11	18	2791	250	8.96	5.74	13.71
Apodemus sylvaticus[(e)]	9	15	1736	50	2.88	1.01	7.91
Apodemus agrarius[(f)]	7	10	889	46	5.17	2.60	10.04
Microtus agrestis[(g)]	6	7	364	14	3.85	3.21	4.61
Rattus spp.[(h)]	6	7	250	39	15.60	5.19	38.41
Soricidae[(i)]	5	10	116	1	0.86	0.07	9.32
Mus spp.[(j)]	5	6	122	25	20.49	6.37	49.39
Apodemus spp.[(k)]	3	4	771	60	7.78	4.72	12.57
Microtus subterraneus[(l)]	3	3	21	1	4.76	0.18	57.87
Micromys minutus[(m)]	2	2	5	0	0.00	0.00	52.18
Arvicola spp.[(n)]	1	3	415	9	2.17	0.66	6.87
Small rodents[(o)]	1	1	465	90	19.35	16.01	23.20
Alces alces[(p)]	1	1	427	5	1.17	0.49	2.78
Apodemus uralensis[(q)]	1	1	186	3	1.61	0.52	4.88
Vulpes vulpes[(r)]	1	1	125	3	2.40	0.78	7.18
Neomys anomalus[(s)]	1	1	1	0	0.00	0.00	97.50
Pitymus spp.[(t)]	1	1	1	0	0.00	0.00	97.50

(a) Prevalence estimates cannot be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study; (b) Aberle et al., 1999, Artois et al., 2007, Asikainen et al., 2000, Augot et al., 2006, Augot et al., 2008, Avsic-Zupanc et al., 2007, Deter et al., 2008, Escutenaire et al., 2000a, Escutenaire et al., 2001, Escutenaire et al., 2002, Essbauer et al., 2006, Golovljova et al., 2002, Heroldová et al., 2010, Heyman and Saegerman, 2009, Heyman et al., 2009c, Kallio et al., 2007, Kallio-Kokko et al., 2006, Klempa et al., 2005, Klingström et al., 2002, Korva et al., 2009b, Nemirov et al., 2010, Olsson et al., 2003, Olsson et al., 2005, Plyusnina et al., 2009, Razzauti et al., 2009, Sauvage et al., 2002, Sironen et al., 2002, Song et al., 2004, Tersago et al., 2008, Weidmann et al., 2005 ; (c) Aberle et al., 1999, Artois et al., 2007, Avsic-Zupanc et al., 2000, Deter et al., 2008, Essbauer et al., 2006, Golovljova et al., 2002, Heroldová et al., 2010, Jakab et al., 2007, Kallio-Kokko et al., 2006, Klempa et al., 2005, Korva et al., 2009b, Nemirov et al., 2004, Papa et al., 2000, Sibold et al., 2001, Sironen et al., 2002, Song et al., 2004, Weidmann et al., 2005; (d) Aberle et al., 1999, Artois et al., 2007, Deter et al., 2008, Golovljova et al., 2002, Heroldová et al., 2010, Heyman et al., 2002a, Jakab et al., 2008, Kallio-Kokko et al., 2006, Klingström et al., 2002, Korva et al., 2009a, Pejcoch et al., 2003, Reusken et al., 2008, Schmidt-Chanasit et al., 2010, Song et al., 2004, Weidmann et al., 2005; (e) Aberle et al., 1999, Artois et al., 2007, Avsic-Zupanc et al., 2000, Deter et al., 2008, Escutenaire et al., 2000a, Heroldová et al., 2010, Heyman and Saegerman, 2009, Heyman et al., 2009c, Jakab et al., 2007, Kallio-Kokko et al., 2006, Klingström et al., 2002, McCaughey et al., 1996, Sironen et al., 2002, Weidmann et al., 2005; (f) Avsic-Zupanc et al., 2000, Golovljova et al., 2002, Jakab et al., 2007, Klempa et al., 2005, Korva et al., 2009b, Laakkonen et al., 2006, Nemirov et al., 2004, Sibold et al., 2001, Song et al., 2004; (g) Artois et al., 2007, Avsic-Zupanc et al., 2007, Golovljova et al., 2002, Korva et al., 2009a, Schmidt-Chanasit et al., 2010, Sironen et al., 2002, Weidmann et al., 2005; (h) Filipe et al., 1991, Golovljova et al., 2002, Heyman et al., 2004, Heyman et al., 2009a, McCaughey et al., 1996, Papa et al., 2000; (i) Golovljova et al., 2002, Heroldová et al., 2010, Heyman et al., 2009c, Sironen et al., 2002, Song et al., 2007, Weidmann et al., 2005; (j) Aberle et al., 1999, Heroldová et al., 2010, McCaughey et al., 1996, Sironen et al., 2002, Song et al., 2004, Weidmann et al., 2005; (k) Artois et al., 2007, Plyusnina et al., 2009, Schlegel et al., 2009; (l) Heroldová et al., 2010, Jakab et al., 2008, Korva et al., 2009a; (m) Heroldová et al., 2010, Sironen et al., 2002; (n) Artois et al., 2007, Charbonnel et al., 2008, Deter et al., 2008; (o) Heyman et al., 2002b; (p) Ahlm et al., 2000; (q) Heroldová et al., 2010; (r) Escutenaire et al., 2000b; (s) Song et al., 2007; (t) Song et al., 2004.

6.1.5. Information on the occurrence and geographical distribution of Hantavirus and hosts

Agent

Hantaviruses have been discovered to circulate in Europe, Asia, Americas and Africa. Our knowledge on the occurrence of Hantavirus in Europe, Norway and Switzerland, considering data both on human cases and animal virus detection, demonstrated the virus circulation in all the countries excluding Malta and Cyprus where no data were found (this however does not imply that the infection is not present in these countries) .

Distribution of Hantavirus subtypes, by country is shown in Table 32, where tested animal species on which viral characterization was carried out are also reported. Details on each country can be found in Annex B.

Table 32. Geographic distribution of Hantavirus subtypes detected by active data collection in animals, based on the systematic literature review of publications from 2000 through 2010[(a)].

Country	Tested animal species	Identified subtypes
Austria	*Apodemus flavicollis*[(b)], *Mus* spp.[(c)], *Myodes glareolus*[(d)]	PUUV, HNTV
Belgium	*Apodemus sylvaticus*[(e)], Fox[(f)], *Microtus arvalis*[(g)], *Myodes glareolus*[(h)],Small rodents[(i)]	PUUV, TULV, SEOV
Czech Republic	*Apodemus sylvaticus*[(j)], *Apodemus uralensis*[(k)], *Microtus arvalis*[(l)], *Microtus subterraneus*[(m)], *Mus* spp. [(n)], *Myodes glareolus*[(o)]	DOBV, TULV
Denmark	*Apodemus agrarius*[(p)], *Myodes glareolus*[(q)]	PUUV, SAAV
Estonia	*Apodemus agrarius*[(r)], *Myodes glareolus*[(s)]	PUUV
Finland	*Apodemus agrarius*[(t)], *Myodes glareolus*[(u)]	PUUV, SAAV
France	*Arvicola* spp. [(v)], *Microtus arvalis*[(w)], *Myodes glareolus*[(x)], *Rattus* spp. [(y)]	PUUV, TULV, SEOV
Germany	*Apodemus flavicollis*[(z)], *Apodemus* spp. [(aa)], *Microtus agrestis*[(ab)], *Microtus arvalis*[(ac)], *Myodes glareolus*[(ad)]	DOBV, PUUV, TULV
Greece	*Apodemus flavicollis*[(ae)]	DOBV
Hungary	*Apodemus agrarius*[(af)], *Apodemus* spp. [(ag)], *Microtus arvalis*[(ah)], *Myodes glareolus*[(ai)]	DOBV, PUUV, TULV, SAAV
Italy	*Apodemus flavicollis*[(aj)], *Myodes glareolus*[(ak)]	DOBV, PUUV
Netherlands	*Microtus arvalis*[(al)]	TULV
Norway	*Myodes glareolus*[(am)]	PUUV
Poland	*Apodemus agrarius*[(an)], *Apodemus flavicollis*[(ao)], *Microtus arvalis*[(ap)], *Mus* spp. [(aq)], *Myodes glareolus*[(ar)]	PUUV
Slovakia	*Apodemus agrarius*[(as)], *Apodemus flavicollis*[(at)]	DOBV
Slovenia	*Apodemus agrarius*[(au)], *Apodemus flavicollis*[(av)], *Microtus agrestis*[(aw)], *Microtus arvalis*[(ax)], *Microtus subterraneus*[(ay)], *Myodes glareolus*[(az)]	DOBV, PUUV, TULV, SAAV
Sweden	*Alces alces*[(ba)], *Myodes glareolus*[(bb)]	PUUV
Switzerland	*Neomys anomalus*[(bc)], Soricidae[(bd)]	Seewis virus (SWSV)
United Kingdom	*Apodemus sylvaticus*[(be)], *Mus* spp. [(bf)], *Rattus* spp. [(bg)]	SEOV virus (strain R22), HNTV

(a) The information presented in this table is affected by sampling different animal species and, therefore, cannot be considered as a valid estimate of the geographic distribution of subtypes; (b) Aberle et al., 1999; (c) Aberle et al., 1999; (d) Aberle et al., 1999; (e) Escutenaire et al., 2000a, Heyman and Saegerman, 2009, Heyman et al., 2009c, Klingström et al., 2002; (f) Escutenaire et al., 2000b; (g) Heyman et al., 2002a, Klingström et al., 2002; (h) Escutenaire et al., 2000a, Escutenaire et al., 2001, Escutenaire et al., 2002, Heyman and Saegerman, 2009, Heyman et al., 2009c, Klingström et al., 2002, Tersago et al., 2008; (i) Heyman et al., 2002b, Heyman et al., 2009a; (j) Heroldová et al., 2010, Weidmann et al., 2005; (k) Heroldová et al., 2010; (l) Heroldová et al., 2010; (m) Pejcoch et al.,2003; (n) Weidmann et al., 2005; (o) Pejcoch et al., 2003; (p) Nemirov et al., 2004; (q) Asikainen et al., 2000, Sironen et al., 2002; (r) Golovljova et al., 2002; (s) Golovljova et al., 2002; (t) Laakkonen et al., 2006; (u) Kallio et al., 2007, , Razzauti et al., 2009; (v) Charbonnel et al., 2008; (w) Artois et al., 2007, Deter et al., 2008; (x) Artois et al., 2007, Augot et al., 2006, Augot et al., 2008, Deter et al., 2008, Sauvage et al., 2002; (y) Heyman et al., 2004; (z) Essbauer et al., 2006; (aa) Schlegel et al., 2009; (ab) Schmidt-Chanasit et al., 2010; (ac) Schmidt-Chanasit et al., 2010; (ad) Essbauer et al., 2006; (ae) Papa et al., 2000, Papa et al., 2001; (af) Jakab et al., 2007; (ag) Plyusnina et al., 2009; (ah) Jakab et al., 2008; (ai) Plyusnina et al., 2009; (aj) Kallio-Kokko et al., 2006; (ak) Kallio-Kokko et al., 2006; (al) Reusken et al., 2008; (am) Lundkvist et al.,1998; (an) Song et al., 2004; (ao) Song et al., 2004; (ap) Song et al., 2004; (aq) Song et al., 2004; (ar) Song et al., 2004; (as) Sibold et al., 2001; (at) Sibold et al., 2001; (au) Avsic-Zupanc et al., 2000, Korva et al., 2009b; (av) Avsic-Zupanc et al., 2000, Korva et al., 2009b; (aw) Korva et al., 2009a; (ax) Korva et al., 2009a; (ay) Korva et al., 2009a; (az) Avsic-Zupanc et al., 2007, Korva et al., 2009b; (ba) Ahlm et al., 2000; (bb) Nemirov et al., 2010, Olsson et al., 2003, Olsson et al., 2005; (bc) Song et al., 2007; (bd) Song et al., 2007; (be) McCaughey et al., 1996; (bf) McCaughey et al., 1996; (bg) McCaughey et al., 1996.

Hosts
See annex F on distribution of hosts

6.2. Proposal for data collection on Hantavirus in animals

The application of criteria for the selection of animal species to be proposed for data collection can be summarized as follows (the application of criteria can be seen in detail in Annex C).

Rodent reservoir hosts play the key role in Hantavirus maintenance and in transmission to humans. As a consequence, data collection in rodents would be useful to detect Hantavirus frequency and subtype composition even before human cases of infection are reported (Heyman and Saegerman, 2009). There is a strong positive correlation between bank vole numbers and the risk of humans acquiring PUUV-HFRS. For example, in Finland, where many human cases are reported, bank vole populations show multiannual cyclic patterns of 3-4 years with increase, peak, and decline/low phase, this information is good predictor of changing in risk (Olsson et al., 2010). In Sweden, bank vole abundance alone explained >70% of the variation in seasonal disease incidence. During a rapid population growth, either in cyclic increase phase or due to masting, one should expect a simultaneous build-up of recently Hantavirus infected rodent and a virus diffusion that cause a rapid frequency-dependent transmission of Hantavirus across the rodent population, and subsequently increase risk to humans (Olsson et al., 2010).

Among drawbacks of data collection in rodents, in comparison with data collection from domestic animals, rodent trapping and subsequent examination are much more laborious. Moreover, rodent population abundance and composition undergo small scale spatial variations, and the association between Hantavirus subtypes and rodent species would reduce information from data collection only on certain rodent species.

Dogs and cats have been the objects of previous studies on Hantavirus and can be in contact with rodents and with rodent-contaminated environments (Bennett et al., 1990, Nowotny, 1994; Nowotny et al., 1994; Malecki et al., 1998; Escutenaire et al., 2000b; Dobly et al., 2011). Advantages of dogs and cats over other species include widespread distribution in association with humans with which share habitat frequentation. The infection by Hantavirus does not cause clinical signs, but these species produce specific antibodies. Diagnostic tests for use in humans have been adapted for dogs and cats, and data on accuracy when used in animals are lacking. Furthermore, data on duration of antibody response are not available. In a study in Belgium, prevalence was greater in cats than in dogs (16.9 vs 4.9%). Blood serum collection is easy to perform and animals are routinely tested also for other agents. Gathering information on exposure to habitat and movements is relatively easy, also because the anamnesis of domestic animals is easily available by interviewing owners.

Cattle have been tested for antibodies against Hantavirus in the Czech Republic (Daneš et al., 1992) and prevalence was 1.4%.

Wild animals, such as deer, hare, foxes are less available for sampling, and harmonised data collection is difficult in comparison with domestic animals. However, they are exposed to the agent and were found positive for antibodies against Hantaviruses in serological studies (Zeier et al., 2005).

Based on the application of the criteria mentioned above, we decided to consider as candidate to be involved in data collection: rodents, dogs and cats. Due to the absence of clinical signs in animals, only active data collection is proposed. Proposals for data collection on Hantavirus in animals are summarised in Table 33.

6.2.1. Data collection on Hantavirus in rodents

Specifications on active data collection on Hantavirus in rodents

Subpopulations to be covered

Rodents that live in areas that are frequented by humans should be covered. Therefore, data collection should include wild rodents in natural areas frequented mostly for recreational or occupational reasons, and also synanthropic rodents living in urban and rural areas.

Agents and possible subtypes

All subtypes circulating in Europe should be considered. For this reason, all species of rodents present at a location should, ideally, be sampled.

Sampling context and design, sampling stage

In active data collection on wild rodents, land areas should be the primary sampling units. Accordingly, each country could be divided in 20 by 20 km squares. One square should then be randomly selected in each first order administrative area, retaining only those including wooded areas frequented by people. If squares with such chararcteristics are not selected, other squares should be selected, in each areas, until a suitable one is found. Availability of personnel in protected areas could also be a factor in the inclusion of locations for data collection. Rodents should be trapped overnight by using live traps, during monthly sessions in spring and summer. In Northern Europe, rodents may access human settlements in autumn, and trapping can also be carried out in this season at those locations.

For data collection on synanthropic rodents, such as rats, trapping sites should be identified in rural areas (near farms) and in urban and suburban areas.

Type of specimen to be taken and sampling technique

Captured rodents should be anesthetized for the collection of blood and then euthanized for the collection of lungs and other organs (to be preserved frozen) (http://www.cdc.gov/rodents/).

Diagnostic methods to be used

An ELISA for the detection of antibodies against Hantavirus can be used as diagnostic method. ELISA-positive individuals should be tested by RT-PCR on lungs. Sequencing of PCR products would allow the identification of Hantavirus subtype.

Data submission

Geographic coordinates of trapping locations should be provided. Data on the number and species of trapped animals, and number and species of positive animals, should be submitted in order to analyze Hantavirus infection in the different geographical areas.

Environmental monitoring of Hantavirus

Hantavirus transmission is affected by the population dynamics of reservoir hosts that, in turn, depend on environmental factors such as seed production by trees (oaks, beech trees) as a source of food, land characteristics, and climate. Increasing temperatures have been proposed as a factor favouring Hantavirus transmission in certain areas (Linard et al., 2007; Carver et al., 2010; Olsson et al., 2010). For these reasons, the collection of data on wild rodent population abundance and on climate and food availability for rodents should be considered together with direct data collection on animals. By the appropriate analysis and interpretation of these data, trends in Hantavirus in natural cycles could be predicted and the association among the involved factors could be better studied.

6.2.2. Data collection on Hantavirus in dogs and cats

Specifications on active data collection on Hantavirus in dogs and cats
Subpopulations to be covered

Dogs and cats with access to outdoor environment, or sites where rodents are present, should be included in data collection.

Agents and possible subtypes

All Hantavirus circulating in Europe would be of interest, keeping in mind that PUUV is most often involved in human disease

Sampling context and design, sampling stage

Dogs and cats can be sampled either by random selection from anagraphic lists or, more realistically, by convenience sampling based on owner compliance. Sera collected for other infections can be used. Stratification at the level of second order administrative area is proposed.

Type of specimen to be taken and sampling technique

Blood serum.

Diagnostic methods to be used

ELISA or IFA tests for the detection of antibodies against Hantavirus. Tests for the identification of antibodies against different subtype would be useful, but ELISA and IFA are not perfectly accurate for the purpose of Hantavirus classification, while FRNT is laborious (see section).

Data submission

Greatest prevalence of antibodies against Hantavirus was previously found in dogs from wooded geographic areas (Dobly et al., 2011). Information on vegetation cover in second order administrative areas should be reported. Since information on duration of antibody reponse in dogs and cats is not available, the month of blood collection should be reported.

Table 33. Summary of proposals for active data collection on Hantavirus in animals.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimes	Diagnosis
Rodents	Wild rodents in natural areas frequented by humans, synanthropic rodents living in urban and rural areas	Live trapping and euthanasia, in 20 by 20 km land units.	Stratified by administative area	Blood serum, lungs	ELISA, RT-PCR on lungs
Dogs and cats	Animals having access to outdoors	Convenience sampling based on owner compliance		Blood serum	ELISA, IFA

6.3. References on Hantavirus

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7. CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS

The Crimean-Congo hemorrhagic fever (CCHF) virus belongs to the genus *Nairovirus* of the family Bunyaviridae. It causes a severe disease in humans, with high fatality rates, and has been described in many countries in Africa, Asia, Eastern Europe and the Middle-East (Ergonul, 2006; Hoogstraal, 1979). In Europe, serious outbreaks have been reported in Bulgaria during the period 1953-1973. More recently (since 1999 - to date) cases or outbreaks have been recorded in Bulgaria again, in Turkey, as well as in Kosovo, Albania and Greece (Ahmed et al., 2009).

7.1. Inventory of available data

7.1.1. Transmission cycle of CCHFV

CCHF virus (CCHFV) circulates in nature in an enzootic tick–vertebrate–tick cycle and does not cause disease in animals other than experimentally infected newborn mice. The number of vertebrates species involved in CCHF epidemiology is extensive, and the virus has been isolated from numerous domestic and wild animals, including domestic ruminants, hares, hedgehogs, mice and even dogs (Causey et al., 1970; Gear, 1988; González et al., 1988; Shepherd et al., 1987a,b; Hoogstraal, 1979). However, the role of these species in the maintenance and transmission of the virus is complex. Based on the current knowledge, wild mammals such as rodents, hedgehogs, hares, and possibly some birds, are considered the principal amplifying hosts of CCHFV (EFSA, 2010).

It has been suggested that CCHFV infection and viraemia most frequently occur in large mammals, because they are the preferred hosts of adult *Hyalomma* ticks (Nalca and Whitehouse, 2007). However, the viraemic periods in ruminants appear to be short, and this reduces the probability of transmission of the virus to other hosts (EFSA, 2010). Most birds are refractory to infection, but they can spread CCHFV-infected ticks along migration routes (Hoogstraal et al., 1961).

CCHFV can be transmitted to humans by tick bites, contact with crushed infected ticks, contact with viraemic tissues/organs of infected wild or domestic animals, and through person-to-person transmission by contact with infectious blood or body fluids (Hoogstraal, 1979). The studies regarding the vector competence for CCHFV have consistently shown that Ixodid (hard) ticks, particularly members of the genus *Hyalomma*, are highly susceptible to CCHFV infection and that infected ticks can transmit this virus by bite. Indeed, occurrence of CCHF closely approximates the known world distribution of *Hyalomma* spp. ticks (Hoogstraal, 1979). Exposure to these ticks represents a major risk factor for contracting the disease, but a two-host tick cycle needs to be completed. Larvae mostly feed on small mammals and ground-feeding birds, and adult ticks feed on large mammals, e.g. livestock and/or wildlife species (Turell, 2007). The link of the virus with the ticks and the need that ticks have for animal hosts in order to complete their life cycle and survive, clearly prove that vertebrates are essential as source of blood for vector ticks which will be the final responsible of the disease transmission (Nalca and Whitehouse, 2007).

In addition to trans-stadial transmission, allowing the virus to be maintained between different tick stages, some infected female ticks may be able to transmit the virus to their eggs. When these eggs hatch, the larvae are already infected and able to transmit the virus by bite during their first blood meal (Turell, 2007). Moreover, ticks are able to become infected with the virus when co-feeding with virus-infected ticks on the same vertebrate host, even if the vertebrate does not develop a detectable viraemia (Turell, 2007).

Recent studies have demonstrated that CCHF may be re-emerging in some areas of eastern Europe and Near East. Serological surveys carried out in western Europe did not report the presence of serologically positive people, however, as the disease has a very severe course, it is unlikely that clinical cases might have been overlooked. We must thus conclude that the pathogenic form of the virus does not yet occur in most of the western Mediterranean basin, although the main tick vector is present and abundant. Actually we do not know yet in details which could be the key regulatory factor

in the presence or absence of active foci of the disease, nor the factor regulating the virus circulation in ticks and reservoir populations.

Serious concern exists about the probable spread of the disease, because the tick is common in sites with a hot and dry climate, and the global warming and ecological changes may favour a wider distribution of the vectors. Surveillance on vectors, animals, and humans should be implemented or intensified in areas around the Mediterranean basin, and should be coordinated at national and international level.

Recent analysis of the ongoing epidemic of CCHF in Turkey revealed that climate changes are not behind the increase in disease incidence. However, a critical fragmentation of the habitat seems to cause such spread. Recent social changes, like abandonment of the lands, have provided the adequate culture for the overpopulations of the main reservoir hosts, as well as of the tick vector. Simulations carried out on the epidemic wave of the disease in Turkey revealed that the infected ticks "were there" and that the changes in the habitat were responsible of a higher contact rate between humans and infected ticks. Data from the epidemic in Turkey are not compatible with a travelling wave of infected ticks across the country. As a summary, while the climate has an obvious effect on the distribution of the tick vector, it may not be considered responsible of the recent outbreaks.

7.1.2. Diagnosis of CCHFV in animals.

Clinical signs in animals caused by CCHFV

CCHF infection in animals is not characterized by clinical signs. The dynamic of CCHF infection is well documented in humans, but only few data exist for animal infection (OIE, 2010).

Laboratory diagnosis of CCHF in animals

CCHF is diagnosed by combining direct and indirect methods (viral isolation, detection of viral genome or antigens, detection of specific antibodies). CCHF is a notifiable disease to the World Organisation for Animal Health (OIE), but no specific recommendations or guidelines are currently available for animal testing. The virus is considered a Biosafety Level 4 (BSL-4) agent in most non-endemic areas, and a BSL-3 agent in several endemic countries, so adequate containment facilities are needed for laboratory and animal work with live virus (Nalca and Whitehouse, 2007; OIE, 2010).

Direct laboratory diagnosis

The most sensitive direct diagnostic method is the isolation by intracerebral inoculation of suckling mice; inoculation of susceptible cell lines (VERO, CER) may also be used.

Immunofluorescence test (IFA) may be used to detect the virus in infected cells or tissues (Shepherd et al, 1986).

Reverse transcriptase-polymerase chain reaction (RT-PCR) has also been developed for diagnosis in humans (Burt et al., 1998). Viral RNA can be extracted from potentially infectious material by using a detergent-based protocol, which makes it non-infectious, so subsequent RT-PCR can be easily performed (Zeller, 2007). A RT-PCR commercial kit also exists (target: S segment RNA), whose sensitivity and specificity are reportedly 100% (ECDC, 2008).

Indirect laboratory diagnosis

The ELISA test is the most used technique for CCHFV antibody detection and is reportedly more sensitive than IFA (Burt et al., 1993). A sandwich ELISA is generally used to detect IgG, and an immunocapture ELISA for IgM (Lotfollahzadeh et al., 2011; Garcia et al., 2006; Khan et al., 1997). For IgG detection, sandwich ELISA with capture of the CCHFV antigen in plates coated with a CCHFV MHIAF (specific CCHFV antibody) have been extensively used in animal serosurveys. Domestic and wild animals have also been tested by competitive ELISA using a rabbit anti-CCHFV peroxidase conjugate (Zeller, 2007).

Serological diagnostic methods have been developed using either inactivated virus or extracts from infected suckling mouse brain (Burt et al., 1994). Since CCHFV is highly pathogenic for humans and the production of native antigen is difficult, recombinant nucleoprotein of the CCHFV have been produced (Garcia et al., 2006), to allow the diagnosis in laboratories with biosafety specifications below BSL-4 level (Chinikar et al., 2008).

Reverse passive hemagglutination inhibition (RPHA) is also used for diagnosis (Shepherd et al., 1989; Swanepoel et al., 1983; Hassanein et al., 1997).

According to ECDC (2008), there are commercial indirect tests available, namely a CCHF IgG Sandwich ELISA and an IFA test based on recombinant antigens GPC and N.

7.1.3. Data collection on CCHFV in animals by competent authorities of EU countries, Norway and Switzerland.

In this section, past and current data collection on CCHFV in animals is EU countries, Norway and Switzerland are summarised, based upon information gathered through the questionnaire submitted to members of the Task Force on Zoonoses data collection.

Summary of results from questionnaires

Eleven MSs (Denmark, Estonia, Finland, France, Hungary, Italy, Latvia, Portugal, Romania, Slovakia, Sweden), plus Switzerland and Norway responded to the questionnaire regarding CCHF. CCHF is notifiable in animals in four of these countries: Italy, Finland, Latvia, and Romania. Past or current data collection on CCHFV in animals was reported in Romania only.

Country by country results

The main results of the questionnaire on active data collection on CCHF in animals are summarized in Table 34, while country by country results are reported in Annex A.

Table 34. Summary of results of the questionnaire on active data collection on CCHFV in animals.

Country	Time span	Animal species	Diagnosis	National Reference Laboratory
Denmark				National Veterinary Institute Technical University of Denmark
France				*Centre National de Référence des Arbovirus*, Pasteur Institute
Hungary				The Central Agricultural Office
Italy				Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise *"G. Caporale"*
Romania	2010-present	cattle and sheep	Indirect IFAT	National Reference Laboratory for vectors of animal diseases and zoonoses

[a] No data collection activities nor reference laboratory were reported in the questionnaires from Estonia, Finland, Latvia, Norway, Portugal, Slovakia, Sweden, Switzerland. No data collection activities were reported in the questionnaires from Denmark, France, Italy.

Comments on the questionnaires results

CCHF is a notifiable disease to the World Organisation for Animal Health (OIE, 2010), according to the requirements on notification of diseases set out in Chapter 1.1.2. of the OIE Terrestrial Animal Health Code. It is an OIE listed disease, but not yet described in the Terrestrial Code and in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

The relevance of this disease for OIE notification is not related to the consequences of its spread within the animal population, but rather to the risk posed by its zoonotic potential. EU MSs, as all OIE Member Countries, agree upon OIE statute to fulfil their international reporting obligations as laid down in the Terrestrial Code, making national and International notification of CCHF cases to be mandatory for all member countries as from 1 January 2005 (immediate notification, within 24 hrs, and follow-up reports).

The fact that only few MSs, who responded the questionnaire, consider CCHF as notifiable disease in the respective countries is a matter of concern as regards the harmonization process of disease reporting within the EU.

7.1.4. Review of data collection on CCHFV in animals.

Due to the scarcity of data collection activities on CCHFV in EU and Norway and Switzerland, a systematic literature review of publication could not be carried out for this agent. Alternatively, here we present results of a narrative literature review on CCHFV in animals on a wider geographical scale, with particular focus on the role of animals in CCHF epidemiology. This review will serve as a source of scientific information to generate proposals of data collection in animals, within the objectives of this report.

As regards the role of animals in CCHF epidemiology, two bibliographic references of general interest are the comprehensive reviews published by Hoogstraal (1979) and Ergonul and Whitehouse (2007).

Domestic animal species

Seroepidemiological surveys in CCHF endemic areas demonstrated that a number of domestic animals become infected with the virus, and, in most of the cases, antibodies against CCHFV have been detected (Khan et al., 1997).

Livestock is believed to play an important role in CCHF ecology (Chumakov et al., 1970; Nalca and Whitehouse, 2007). CCHFV has been detected in domestic cattle, horses, donkeys, sheep, goats and camels from various parts of Europe, Asia, and Africa (Watts et al., 1989). Their role as reservoir depends on the level of viraemia during infection, as only viraemia above a certain level is considered sufficient to infect feeding ticks (Nalca and Whitehouse, 2007).

Domestic ruminants have been identified as important animal species involved in CCHF infections, can develop demonstrable viraemia and infect ticks (Hoogstraal, 1979; Shepherd et al, 1991). Cattle and sheep, in particular, present high prevalence of CCHFV antibodies (Fourie et al., 1996).

Experimental infections in horses showed low levels of infection and absence of viraemia, while neutralizing antibodies were present at high levels in some animals (Milyutin et al., 1969).

Antibodies to CCHFV have been detected in pigs from various regions of Europe, Asia, and Africa, but disease and viraemia have not been observed, so a limited role of this species in the epidemiology and ecology of CCHF is suspected (Ergonul, 2006).

In the case of companion animals, low levels of antibodies have been detected in domestic dogs and experimental studies in cats found out that they are refractory to infection (Saidi et al., 1975; Shepherd et al., 1987a).

European Union countries (MSs)

CCHF was first reported in Bulgaria in the early ‘50s, with more than 1,000 human cases reported during the period 1953-1974. Most CCHF clinical cases and highest seropositivity were reported amongst persons involved with livestock (mainly cattle) raising activities. Cattle were the principal hosts for *H. marginatum* ticks (90% of the ticks collected during a survey in the country). In the same survey, the prevalence of CCHFV antibodies in sheep sera was lower than in cattle (28% and 47% respectively), while horses unexpectedly presented the highest prevalence (82%) (Hoogstraal, 1979). Most animal cases, in the Bulgarian disease-endemic areas, were reported from Plovdiv and Pazardgik (central Bulgaria), Haskovo and Kardgali (southeastern Bulgaria), Shumen (northeastern Bulgaria), and Burgass (eastern Bulgaria) (Papa et al., 2004).

Another country at risk is Greece, where an autochthonous human CCHF case has been recently diagnosed (Maltezou et al., 2009). Although CCHF virus (AP-92 strain) was isolated from *Rhipicephalus bursa* ticks collected from goats in northern Greece (Papadopoulos and Koptopoulos, 1978), recent serological data from domestic animals in northern Greece indicate no previous exposure to CCHF. These findings suggest that it is unlikely that these animals, under the present epidemiological situation, play a significant role in disease transmission (Greek Ministry of Agricultural Development, unpublished data; cited in Maltezou et al, 2009). The establishment and maintenance of a CCHF endemic focus requires an environment favouring an efficient contact between competent ticks and animal hosts with relatively high prevalence of infection, and this is not apparently the case of Greece.

In Portugal, during a serological survey for antibodies to CCHFV, 141 goat sera were tested using the plaque reduction neutralization (PRN) and indirect fluorescent antibody (IFA) tests. No positive sera were found (Filipe et al., 1985).

European countries (non MSs)

Over the last decade, climatic, environmental, and anthropogenic factors have driven the expansion of CCHF endemic foci, and outbreaks were recorded in countries neighbouring Greece, e.g. in Kosovo (in 2001) and Turkey (in 2002-2008). Besides the circulation of CCHFV in humans, the virus was detected in animal hosts and ticks in Turkey and in the Balkan countries, where conditions of endemicity have become established.

Almost all European human cases have been reported in the Balkans (Maltezou et al, 2010). CCHF was first reported in Kosovo (then former Yugoslavia) in the '70s, followed by a new outbreak in 1995 and again in 2001, when the largest epidemic in the region occurred (Avsic-Zupanc, 2007). Ticks (*H. marginatum* and *I. ricinus*) collected from cattle after the first epidemic tested positive for the presence of CCHFV. Sera collected from cattle and sheep was tested for the presence of CCHFV antibodies: 32,6% of sheep and 15,4% of cattle were positive (Avsic-Zupanc, 2007). During the 2001 outbreaks, *H. marginatum* adults were collected from cattle in the same villages where human CCHF cases were reported. Ticks were found infected with CCHFV, and the amplicons sequenced showed 98-100% similarity with those of the patients (Duh et al., 2006).

In Albania, CCHF was first signalled in 1986, and again in 2001, coincident with the large outbreak in Kosovo. Although no data are available on the role of livestock in the epidemiology of the disease, similar husbandry techniques as in Kosovo and the competent tick vector were present, make presume a similar epidemiological pattern in Albania as described in Kossovo (Avsic-Zupanc, 2007).

Again in the Balkans, in Serbia, the highest prevalence of antibodies in animal hosts was found in sheep, while lower prevalence was detected in older cattle and calves (Estrada-Peña, personal communication). These data suggest that domestic animals, especially sheep and cattle, should be considered the main hosts for adult ticks, vectors of the virus in this region of Europe and farmers are considered at highest risk of infection.

Finally, in Turkey, an unprecedented and continuous series of CCHF cases has been reported during the past 8 years (2002-2010). This country plays an important epidemiological role as bridge between Europe and Asia. Also in Turkey the disease has been associated to persons involved in animal husbandry: the majority of patients were farmers working in agriculture and/or with livestock keeping. Some studies indicated that in central Anatolia, 74% of cattle were tick-infested, and CCHF antibodies were detected in 79% of sera collected from the same cattle herd (A. Gargili, personal communication; Vatansever et al., 2007).

Non European countries

There are also non-European countries, bordering with European countries (both EU MSs and non MSs) which may play an important role as epidemiological "bridge" in maintaining and/or spreading CCHF.

In the ex-Soviet Republics, the transmission of CCHFV through contact with the blood of infected cows and sheep was reported as an important mechanism of transmission, especially from the countries of Central Asia (Tajikistan and Uzbekistan). Slaughtering infected cattle was identified as a high risk activity (Semashko et al., 1975; Zarubinsky et al., 1976).

Livestock (cattle, sheep, goats, and camels in particular) also play an important role in the transmission of the disease in Iran, where a high number of people live in close contact with animals. Special risk has been observed at the borders of the country with Pakistan and Afghanistan, because the disease can be introduced by the transboundary movements of infected livestock, infested by infected ticks (Saidi et al., 1975; Darwish et al., 1983; Hassanein et al., 1997; Khan et al., 1997; Athar et al., 2003; Chinikar, 2007).

Certain parts of China are known as CCHF endemic areas, particularly the Western region. This region forms the eastern boundary of CCHF endemic region and borders Pakistan, Afghanistan, Tajikistan, and Kyrgyzstan, countries that also have been identified as endemic areas. CCHFV is maintained in nature through cycles of asymptomatic infection in livestock, sheep and goats in particular. CCHFV was also isolated from long-eared jerboas (*Euchoreutes naso*), which suggests that small mammals may also play a role in CCHFV maintenance in this region (Butenko and Karganova, 2007; Saijo, 2007).

As regards the African continent, the first report of CCHFV infection was the detection of antibodies in cattle sera in Tanzania. After the disease was detected in South Africa, it was suggested that the virus had been introduced by translocation of infected tick vectors on migrating birds. However, antibodies to CCHF virus were found in the sera of livestock and wild vertebrates in South Africa, Zimbabwe, and Namibia (Hoogstraal, 1979; Fourie et al., 1996; Causey et al., 1970). In this continent, the preferred domestic hosts for adult *Hyalomma* ticks are large vertebrates such as cattle, sheep, and goats. Antibodies to CCHF were also found in the sera of 6.0% domestic dogs in southern Africa (Shepherd et al., 1987b).

Limited information is available on the presence of ticks and/or seropositive livestock in Madagascar and other islands off the African coast, including the French DOM/TOM. CCHF virus was isolated from *Boophilus* ticks in Madagascar, where seropositive livestock was detected. The virus is not reported in La Reunion, and no information for Mayotte is available (H. Zeller, personal communication).

Wild animal species

Hares, hedgehogs, and mice are considered to be reservoir hosts for CCHFV in some areas of Russia and Bulgaria (Hoogstraal, 1979). In Bulgaria, besides livestock, also little owls, blackbirds and European hares (*Lepus europaeus*) have been identified as the main hosts of the immature stages of the *H. marginatum* tick vectors (Levi, 1972, cit. in Avsic-Zupanc, 2007). In Kazakhstan, 70% of the European hares examined for the presence of specific antibodies to CCHFV were found positive (Semashko et al., 1975).

In Turkey it has been observed that forests, meadows interspersed with pasture, which are inhabited by dense populations of wildlife animals -especially hares and wild boars- surround the villages where CCHF cases occurred (Vatansever et al., 2007).

In South Africa, scrub hares develop a sufficient CCHF viraemia to infect feeding immature Ixodid ticks, but hedgehogs and wild rodents are unlikely to be of importance as maintenance hosts of the virus (Shepherd et al., 1989). Other African wild species, large herbivores in particular, are involved in the CCHF transmission cycle (Horak et al., 1983a,b; Shepherd et al., 1978b; Horak et al., 1983a,b). Therefore, attention should be paid when importing native live African wildlife for introduction in zoological parks, for the potential risk of transmission in case of presence of competent tick vectors or direct accidental contact with animal blood.

Interestingly, there has been only one report of antibody to CCHFV detected from a reptile, a tortoise from Tadzhikistan. However, immature *Hyalomma anatolicum* ticks, a common CCHFV vector, are known to sometimes feed on lizards (Hoogstraal, 1979)

Birds

The studies carried out in birds indicate that several avian species are refractory to the disease and CCHFV infection. It has also been observed that some birds develop antibodies when they are inoculated with CCHFV, but viremia - although present – is not detectable with traditional diagnostic techniques (Zeller et al., 1994a). To clarify the bird infection dynamics, the development of highly sensitive diagnostic tools is therefore needed. Antibodies to CCHFV were observed in some cases in chickens and ducks, in magpie, in blue-helmeted guinea fowls and in a red-beaked hornbill after experimental infection with CCHFV. Moreover, CCHFV has been isolated from ticks infecting several species of ground-feeding birds (Zeller et al., 1994b).

Even though several avian species are not amplifying hosts for CCHFV, they are indirectly involved in the transmission of the disease because they feed infected ticks and can transport them, contributing to the spread of CCHFV along avian migration routes (Hoogstraal et al., 1961).

Antibodies to CCHFV were also detected in ostriches from South African farms; in a limited number of samples examined, 24% of the birds contained antibodies (Capua, 1998). This suggests that

infection in ostriches must be considered a potential transmission route at slaughterhouse level (Swanepoel et al., 1997). As farmers and slaughterhouse workers were affected, the disease was in fact associated with commercial ostrich meat industry in South Africa (Cooper et al., 2004). Due to the fact that ostriches are an efficient host for both the tick vector and CCHFV, EU animal health regulations (Decision 97/183/EC) provide the legislative framework to control introduction of ostriches imported from Africa into European countries for breeding purposes. However, the above regulation does not take into account the importation of ostriches from those European MSs where the disease has been reported.

In Table 35, the results of the narrative review are summarised, illustrating the principal vertebrate species in which CCHFV antibodies have been detected. The countries in which serological studies were performed and the seroprevalence range are reported, when available. Data are not comparable, as in most studies no information on the number of animals tested, the number of positive animals, and the diagnostic tests employed, is available. For this reason, the table has the unique goal to summarize the main vertebrate species studied, and data must be interpreted with caution.

Indeed, further studies are needed to understand the exact role of vertebrate species in the maintenance and transmission of CCHFV.

Based on the current knowledge, a diagram was drawn in order to visualize the transmission cycle of CCHFV and the role of animals (Figure 11). Arrows represent transmission of the virus, whereas animals are divided into three main categories: main reservoir hosts (rodents, hedgehogs, hares), acquiring the infection from infected ticks, and passing it to susceptible ticks (as shown by the two arrows); birds, that are dead-end hosts but can introduce infected ticks (only one, unidirectional, arrow); domestic ruminants and ostriches, that can be infected, develop a short viraemia and have the capacity of infecting ticks and also man, through infected blood or body fluids (dashed arrows). Man may acquire the infection from ticks, and from the contact with viraemic ruminants and ostriches, and develop clinical disease (unidirectional arrows). Ingestion of infected raw milk, although not yet proven as an efficient infection route, could represent a potential risk for consumers as milk from viremic animals infected by other *Bunyaviridae* (Rift Valley fever virus) is a source of infection (Al-Hazmi et al., 2003; WHO, 2010). This schematic representation will be used as a starting point for the selection of animal species for data collection.

Table 35. Summary of the narrative review, describing the principal vertebrate species in which CCHFV antibodies have been detected, the countries in which studies were performed, and the reported seroprevalence range.

Animal species	Country of serological studies	Prevalence range
horses	Bulgaria[(a)], Iraq[(b)]	58.8-82%
goats	Iraq[(c)], Iran[(d)]	36-49%
sheep	Bulgaria[(e)], Kosovo[(f)], Iraq[(g)], Iran[(h)]	28-57.6%
ostriches	South Africa[(i)]	24%
camels	Iraq[(j)]	23.9%
cattle	Bulgaria[(k)], Kosovo[(l)], Turkey[(m)], Iraq[(n)], Iran[(o)], South Africa[(p)], Zimbabwe[(q)]	18-79%
hares	Bulgaria[(r)], Russia[(s)], Kazakhstan[(t)], South Africa[(u)], Zimbabwe[(v)]	14-70%
dogs	Zimbabwe/South Africa[(w)]	6%
wild carnivores	South Africa[(x)], Zimbabwe[(y)], Turkmenistan[(z)], Senegal[(aa)]	1.4%
rodents (squirrels, gerbils, mice, rats, porcupines)	South Africa[(ab)], Zimbabwe[(ac)], Pakistan[(ad)], Iran[(ae)], Egypt[(af)], Mauritania[(ag)], Senegal[(ah)], Russia[(ai)]	0.6-16%

(a) Hoogstraal, 1979; (b) Tantawi et al, 1981, cit. in Nalca & Whitehouse, 2007; (c) Tantawi et al, 1981, cit. in Nalca & Whitehouse, 2007; (d) Saidi et al., 1975; (e) Hoogstraal, 1979; (f) Giglic et al., 1989, cit. in Avsic-Zupanc, 2007; (g) Tantawi et al, 1981, cit. in Nalca & Whitehouse, 2007; (h) Saidi et al., 1975; (i) Shepherd et al., 1987a; (j) Tantawi et al, 1981, cit. in Nalca & Whitehouse, 2007; (k) Hoogstraal, 1979; (l) Giglic et al., 1989, cit. in Avic-Zupanc, 2007; (m) Vatansever et al., 2007; (n) Tantawi et al, 1981, cit. in Nalca & Whitehouse, 2007; (o) Saidi et al., 1975; (p) Swanepoel et al., 1987; (q) Swanepoel et al., 1987; (r) Hoogstraal, 1979; (s) Hoogstraal, 1979; (t) Hoogstraal, 1979; (u) Shepherd et al., 1987b; (v) Shepherd et al., 1987b; (w) Shepherd et al., 1987b; (x) Shepherd et al., 1987b; (y) Nalca & Whitehouse, 2007; (z) Nalca & Whitehouse, 2007; (aa) Nalca & Whitehouse, 2007 ; (ab) Shepherd et al., 1987b; (ac) Shepherd et al., 1987b ; (ad) Darwish et al., 1983; (ae) Saidi et al., 1975; (af) Darwish et al., 1978, cit. in Nalca & Whitehouse, 2007; (ag) Nalca & Whitehouse, 2007; (ah) Nalca & Whitehouse, 2007; (ai) Nalca & Whitehouse, 2007. Note: an unique prevalence value was reported in the case data were available from a single study.

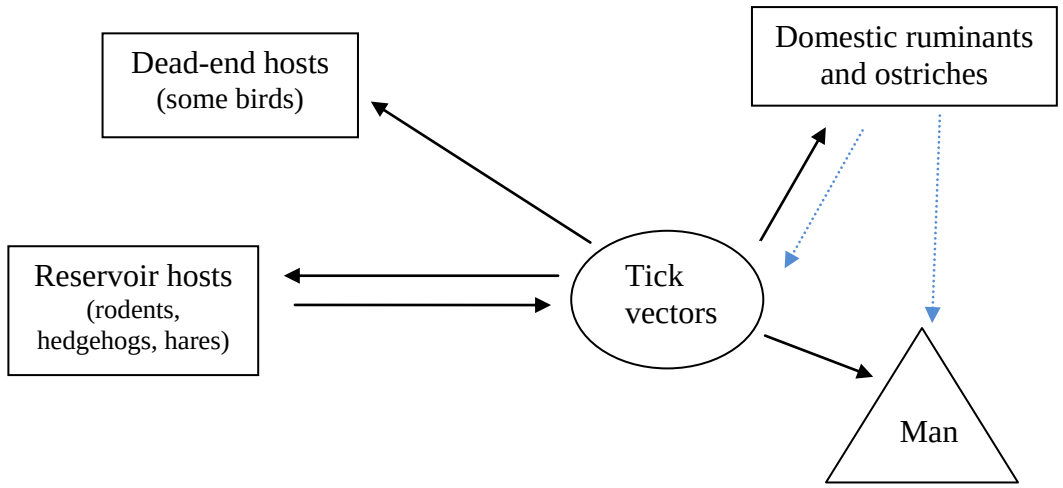

Figure 11. Diagram of the transmission of CCHFV with specific focus on the role of animals.

7.1.5. Information on the occurrence and geographical distribution of CCHF, host and vectors

Agent

CCHFV is transmitted by ticks belonging to the genus *Hyalomma*, particularly by *H. marginatum*. The known occurrence of the disease in Europe, Asia and Africa coincides with the world distribution of *Hyalomma* spp. Other tick species have been found positive to the virus, and all of them exist in Western Europe (Ergonul and Whitehouse, 2007). There is no agreement about why the virus is not present in the whole area of distribution of the tick vector in Europe.

CCHF is endemic in Africa, the Balkans, the Middle East and Asia south of the 50° parallel north, which corresponds with the limit of distribution of the *Hyalomma* tick. Over the last years, sporadic human cases and limited outbreaks are being increasingly reported. CCHF outbreaks have been recorded in Afghanistan (2001 to 2008), Albania (2001), Armenia (2006), Iran (2001-2009), Kazakhstan (2005, 2009), Kosovo (2001 to 2008), Bulgaria (1955 to 2008), Mauritania (2002 to 2003), Pakistan (2001 to 2009), Russia (1999 to 2009) Senegal (2004, with a human imported case in France), South Africa (1989 to 2009), Sudan (2004, 2008), Tajikistan (2002, 2004, 2009), Georgia (2009), Serbia (2001, 2004, 2009), Greece (2008), and Turkey (2003 to 2009). The trend seems to suggest increased human disease incidence with higher case fatality rates (GLEWS, 2009; Hubálek and Rudolf, 2011).

Hosts

The presence of the most important sylvatic host species for CCHFV in EU MSs, Norway and Switzerland is presented in Annex F. Also, population estimates of some domestic animals which can get infected by CCHFV (cattle, goat, sheep and horse) are shown.

Vectors

H. marginatum, also known as the 'Mediterranean *Hyalomma*', may be the main vector of CCHFV in Europe. This tick occurs in the humid Mediterranean climate of northern Africa and southern Europe and in steppe climates eastwards. It is widely distributed in North Africa (Morocco, Tunisia, Algeria). It has been recorded in Portugal, Spain, France, Italy, Greece, Albania, countries of the former Yugoslavia, the Mediterranean islands and Turkey (Estrada-Peña et al., 2004).

CCHFV was isolated from *R. bursa* collected from goats in northern Greece (Papadopoulos and Koptopoulos, 1978). In Europe, this tick is common in every Mediterranean country (Estrada-Peña et al., 2004).

The distribution of the two tick species in Europe is illustrated in Figure 12 and Figure 13.

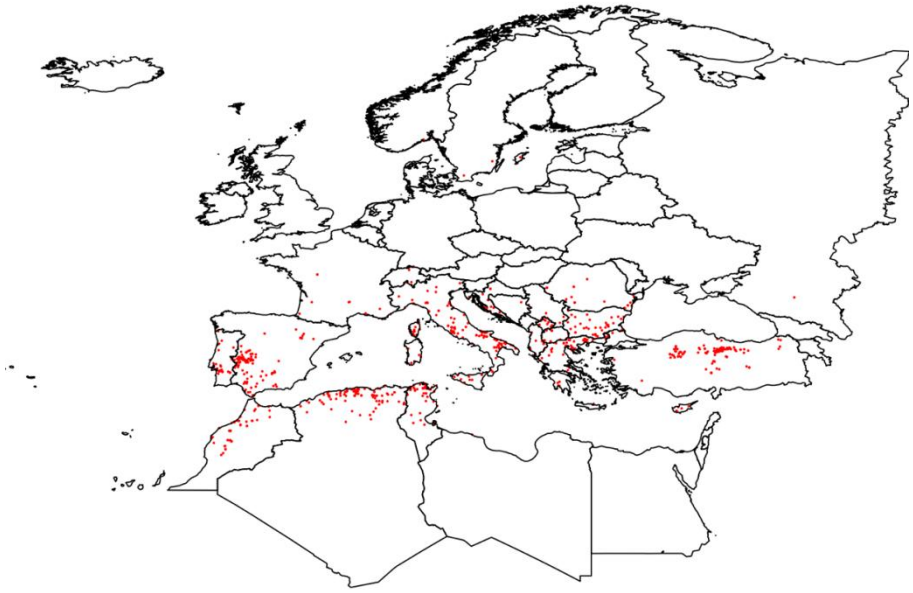

Figure 12. Distribution of *Hyalomma marginatum* in Europe. Dots indicate locations of records of the tick species.

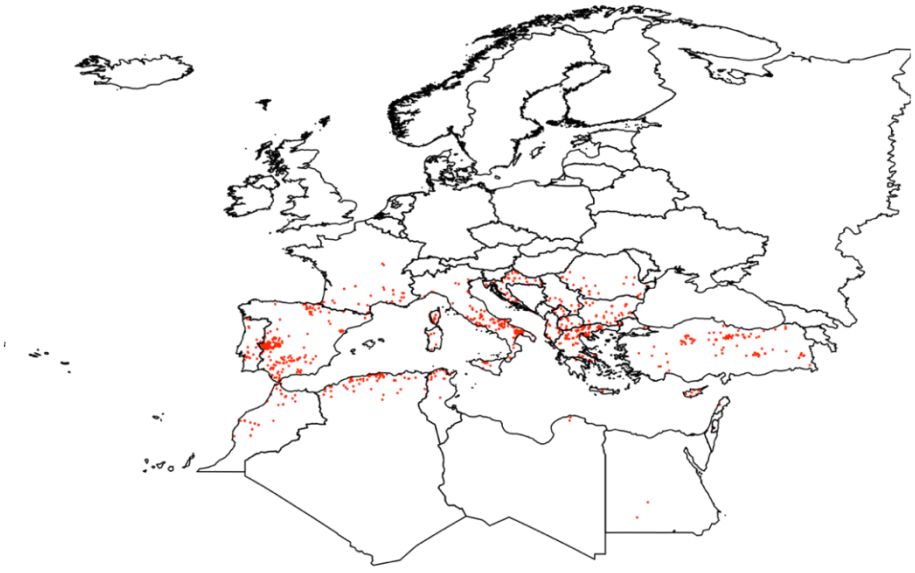

Figure 13. Distribution of *Rhipicephalus bursa* in Europe. Dots indicate locations of records of the tick species.

7.2. Proposal for data collection on CCHFV in animals

To select the animal species to be included in data collection, an initial list of species, that are involved in the transmission cycle of CCHFV, was built based on literature review. A set of criteria was subsequently applied to select species involved in options for data collection. Tables summarising the application of criteria can be found in Annex C. General conclusions that were drawn in this process are listed below.

On the application of the criteria mentioned above, we decided to consider as candidates to be involved in data collection: domestic ruminants, hares, and ostriches. CCHF infection in animals is not characterized by clinical signs so and only active surveillance can be performed.

Particular attention should be paid to the movement of animals from areas where CCHFV was detected and vectors are present. Indeed, the movement of viremic livestock and wildlife species, or of animals which may carry infected ticks, ought to contribute to the spread of the infection (EFSA, 2010).

As mentioned, most birds appear to be refractory to CCHF infection and are not adequate hosts for serological surveys. Horses are considered to play a minor role in the ecology of the disease and can be considered not a priority for surveillance.

Because of the serious risk of spread of CCHF, we propose all those countries in which environmental conditions are adequate for the survival of the vector to be object of surveillance, even if the tick is present only in parts of the country, and the presence of the virus is not yet reported. Here, serological tests on animals should be carried out with the purpose of early detection and rapid alert.

Some countries may be at special risk by CCHFV as they represent the border of the possible spreading way of infected ticks. The evaluation of climate suitability for the tick vector has been studied with a series of climate data from the year 1990, with projection throughout 2020. Undergoing studies suggest that the Balkans are probably the region characterized by the best climate suitability for the vectors in the upcoming years, therefore ticks could increase their abundance and spread over new areas. Special attention must thus to be paid to countries in the Balkans area, since the virus isolation has been already reported in some of these countries, and to the Greece-Turkey border, where CCHF seems to have its natural limit so far. Movements of domestic or wild animals in this area should be then strictly monitored.

In conclusion, the MSs proposed to perform surveillance are: Bulgaria, Cyprus, France, Greece, Italy, Malta, Portugal, Romania, Slovenia, and Spain.

Proposals for data collection on in animals are summarised in Table 36.

7.2.1. Data collection on CCHFV in domestic ruminants

Domestic ruminants can develop demonstrable viraemia and are capable of infecting ticks. They are common hosts of *Hyalomma* adult ticks in the Mediterranean basin. Sero-epidemiological surveys in CCHF endemic areas revealed that a number of domestic animals have been infected with the virus with high prevalence, particularly among cattle and sheep. It is recommended to carry out active surveillance (serological tests at farms and at the slaughterhouse) on cattle and sheep grazed outdoor in natural pastures, to allow an early detection of virus circulation.

Specification on active data collection on CCHFV in domestic ruminants

Subpopulations to be covered

Animals with frequent access to outdoor environment favourable to vectors, i.e. livestock grazed on pastures which represent a suitable habitat for host-seeking *H. marginatum* ticks during the spring-autumn grazing seasons or all-year-round.

Sampling context and design, sampling stage

Sampling may occur both at farm and slaughterhouse level. At the farm level, multistage sampling can be implemented, by randomly selecting herds from a list, and then testing randomly selected individuals within each herd. As a consequence, clustering of observations needs to be taken into account when determining sample size. After the selection of herds, sera can be tested for antibodies against CCHFV (see below) among those that were collected for other disease control programs. Stratification at the first order administrative level, or, if possible, at the second order administrative level is recommended.

Surveillance should be limited to areas at risk within those MSs where the recognized tick vectors (*Hyalomma* spp.) are present and ecological conditions are favourable to the establishment of CCHF foci.

Type of specimen and sampling technique

Blood serum for subsequent antibody detection tests.

Diagnostic methods

Serology is considered the method of choice for diagnosis. A commercial bovine sandwich IgG ELISA is available for cattle. Moreover, a human IgG sandwich ELISA has been used for diagnosis in cattle (Lotfollahzadeh et al., 2011). For other ruminant species, no specific tests exist and ELISA with rabbit or specific anti-species conjugates can be used (Zeller, 2007). Reverse passive hemagglutination inhibition (RPHA) was also used for serological diagnosis in cattle, goats and sheep (Hassanein et al., 1997).

The persistence of IgM antibody activity in sheep and cattle was found to be of shorter duration than in humans, and this may be due to the relative lack of susceptibility of these animals to infection with CCHF virus (Burt et al., 1994).

Data submission

Number of tested and positive animals should be reported. Data relative to animals raised in herd should be accompanied with information on the number of tested herds and herd where at least one positive animal was found. Moreover, a distribution of herd size (number of animals per herd) should be reported. Sample size determination can be found in Annex E.

7.2.2. Data collection on CCHFV in hares

Wildlife may be of special interest for active surveillance on CCHFV by serological surveys. Hares are carriers of the virus because they act as host for immature stages of *Hyalomma* ticks and their role in the maintenance of active foci is well documented in South Africa and Turkey. Active surveillance on hares (*Lepus europaeus*) is recommended, especially in those MSs where recent cases of CCHF in humans have been reported and that export live hares. Nevertheless, this could represent a matter for dispute between MSs for intra-community trade.

Specification on active data collection on CCHF in hares

Subpopulations to be covered

Active data collection on hares should be targeted at hares that are moved from different geographic areas or countries for repopulation or hunting purposes.

Sampling context and design, sampling stage

Hares should be sampled at the time of export from a area.

Serological tests could be performed on live hares which are exported from those MSs where cases of CCHF in humans have been reported. Blood collection for CCHF surveillance can be easily performed

at the same time when sampling for EBHS (European Brown Hare Haemorrhagic Syndrome), when live hares are exported/moved for re-stocking purposes.

Sample size should be based on the minimum prevalence level of the infection to be detected. Unfortunately, no bibliographic data are available on prevalence estimates on hares.

Type of specimen and sampling technique

Blood serum for subsequent antibody detection tests.

Diagnostic methods

Hares are susceptible to infection and have been shown to develop viraemia and antibodies to CCHFV. In experimental infection, the virus persisted in hare blood for 15 days, with the highest titre on day 4 (Hoogstraal, 1979).

No specific tests serological test is available for hares, and ELISA with rabbit or specific anti-species conjugates can be used (Zeller, 2007). Reversed passive hemagglutination inhibition (RPHI) and indirect immunofluorescence (IFA) have also been used to detect antibodies in hares (Shepherd et al., 1987b).

Data submission

Number of exported lots of hares, and number of tested hares, should be reported to account for clustering in statistical analysis.

7.2.3. Data collection on CCHFV in ostriches

Ostriches do not present clinical signs but undergo a viraemia and develop a detectable antibody response. According to Capua (1998), a serological survey on the ostrich population in EU MSs would be interesting in order to gather data on the presence of antibodies to CCHF virus in these birds. In particular, serological tests in ostriches would be recommended in those MSs where recent cases of CCHF in humans have been reported, although this could represent a matter for dispute between MSs with respect to intra-community trade. In case of positive findings in any MSs, the same protecting measures (vector control and quarantine) applied for the importation of birds from South Africa and other endemic countries should be implemented.

Specification on active data collection on CCHFV in ostriches

Subpopulations to be covered

Ostriches with frequent access to outdoor environment favourable to vectors, i.e. questing *H. marginatum* ticks. Sampling should be performed in those countries where recent cases of CCHF have been described.

Sampling context and design, sampling stage

Serological tests could be performed on the ostrich populations in EU MSs, to investigate the presence of antibodies to CCHF virus in these birds in Europe. Sampling could be done at the farm level. The sample design is limited by the likely low number of ostrich farms present in EU MS.

Moreover, it is recommended to test animals that are exported alive (as reproductors) from those MSs where CCHF human cases have been reported.

Type of specimen and sampling technique

Blood serum for subsequent antibody detection tests.

Diagnostic methods

Ostriches undergo viraemia which may last four days. Seroconversion begins on day 5 post-infection and is detectable by day 13 after experimental infection. Indirect ELISA tests can be used for antibody detection in ostriches (Swanepoel et al., 1997).

Data submission

Number of tested and positive animals should be reported. Data relative to animals raised in herd should be accompanied with information on the number of tested herds and herd where at least one positive animal was found. Moreover, a distribution of herd size (number of animals per herd) should be reported.

Table 36. Summary of proposed active data collection activities on CCHFV: animal species, inclusion criteria and stage of sampling.

Animal species	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimens	Diagnosis
Domestic ruminants	Animals frequenting outdoor habitats	At farm	Stratified by administrative area. Limited to those MSs where tick vectors (*Hyalomma* spp.) are present and ecological conditions are favourable to the establishment of CCHF foci	Blood serum	Indirect ELISA test
Hares		At the time of export, from those MSs where human cases of CCHF have been reported			
Ostriches		At farm			
		At the time of export, from those MSs where human cases of CCHF have been reported			

7.3. References on CCHFV

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8. LEISHMANIA

Leishmania infantum is responsible for both cutaneous and visceral forms of leishmaniosis in the Mediterranean basin, with sand flies (*Phlebotomus* spp.) as vectors and dogs as the main domestic reservoir (Pratlong et al., 2004). It has been estimated that at least 2.5 million dogs are infected in southwestern Europe alone (Moreno and Alvar, 2002), and the disease is spreading north into the foothills of the Alps (Ferroglio et al., 2005).

8.1. Inventory of available data on *L. infantum* in animals

8.1.1. Transmission cycle of *L. infantum*.

Dogs are the most important reservoir hosts of *L. infantum*. In wild carnivores the infection has been described in jackals (*Canis aureus*) in Iran, Irak and Kazajstan (Baneth et al., 1998; Mohebali et al., 2005), in foxes (*Vulpes vulpes*) in France (Rioux et al., 1968), Portugal (Abranches et al., 1983), Italy (Bettini et al., 1980) and Spain (Martin-Iniesta,1982; Fisa et al., 1999; Gortazar, 1999), in mangoose, lynx and genet in Spain (Sobrino et al., 2008), in wolf in Iran (Mohebali et al., 2005).

L. infantum is transmitted by phlebotomine sand-fly vectors, in which promastigotes replicate after transformation from intracellular amastigotes taken during the bloodmeal from reservoir hosts. There are numerous sand-fly species, and only a minority of these is competent vectors of canine leishmaniasis (Killick-Kendrick, 1999). *Phlebotomus pernicious* and *P. ariasi* are the most frequently vectors in the Mediterranean basin and *P. perniciosus* is the species with the widest distribution also in new endemic areas (Maroli et al., 2008).

Although sand flies are the only biologically adapted vectors of *L. infantum*, a possible role in transmission for other hematophagous ectoparasites, such as ticks and fleas, has been proposed (Coutinho et al., 2005). However, the significance of transmission to dogs by these arthropods has not been verified in natural conditions.

Congenital transmission of visceral leishmaniasis from mother to offspring is reported in humans and has been experimentally studied in mice. Canine vertical infection was demonstrated experimentally in puppies born from infected male and female beagles, and transmission was assumed to be transplacental; however, investigations in naturally infected dogs resulted into conflicting outcomes (Coutinho and Linardi, 2007; Diniz et al., 2005). In addition to transmission by blood transfusion (de Freietas et al., 2006), dog-to-dog transmission by direct contact has been suggested in the USA in an effort to explain the spread of CanL among kennel foxhounds, in the absence of proven sand-fly vectors (Duprey et al., 2006). Transmission routes other than by sand-fly should be further studied and, at present, it is not known whether they play an important role in the maintenance of canine leishmaniasis (Baneth et al., 2008).

Canine leishmaniasis (CanL) is a complex disease that has a high prevalence of infection, even if it is accompanied by a lower rate of apparent clinical disease (Solano Gallego et al., 2001; Berrahal et al., 1996). Substantial research published recently on the pathogenesis of CanL and immune responses during infection has contributed considerably to our understanding of this complex zoonosis and its epidemiology (Ferroglio et al., 2005; Werneck et al., 2006; Solano Gallego et al., 2001; Leontides et al., 2002, Oliva et al., 2006; Quinnell et al., 2001b). These new insights impact on efforts to prevent and control the disease and its spread into human populations.

The pathogenesis in dogs is critical for the transmission dynamics of *L. infantum* because the capability of dogs to infect the vectors varies in different stages of the infection. Furthermore, the dynamics of antibody response and of the development of clinical signs in dogs need to be taken into account when planning data collection and when interpreting results. For these reasons, the transmission cycle of *L. infantum* and the different stages of infection in dogs are represented in a diagram (Figure 14) and have been the object of a dynamic model (Annex D).

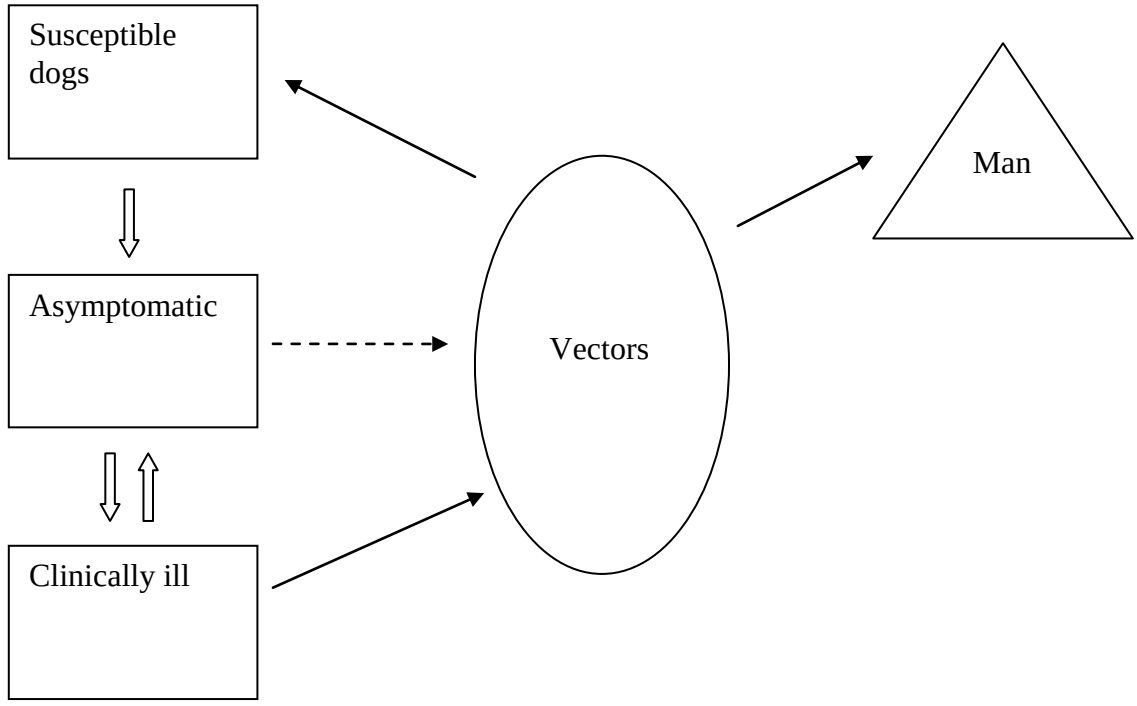

Figure 14. Diagram of the transmission of *L. infantum* with distinction among different stages of infection in dog.

In the diagram, black arrows represent transmission among vectors and animals (including humans, as dead-end hosts). Dogs are divided into three compartments, and transitions among compartments are represented by white arrows. Approximately 90% of dogs are susceptible to *L. infantum*, whereas the remaining 10% are considered as resistant at birth (not represented in Figure 14). Following the bite from an infected vector, susceptible dogs become infected but do not show clinical signs (asyptomatic status). These usually develop a detectable antibody response and may become clinically ill. In fact, some dogs are unable to develop an effective cellular immune response (although they develop a non protective, humoral response), whereas other remain infected for a long period of time but are able to avoid the appearance of lesions and clinical disease. Some of the clinically ill dogs may undergo clinical remittance and go back to the asymptomatic class (as shown by arrows in both directions) (Moreno and Alvar, 2002).

Field studies showed that only a fraction of asymptomatic dogs become clinically ill, and that permanence in the asymptomatic stage is variable (Solano-Gallego et al., 2001; Berrahal et al., 1996; Alvar et al., 2004). A survey from 100 dogs in the island of Mallorca, Spain, indicated that 13% had apparent clinical disease, 26% had antibodies and 63% were found to be positive for *Leishmania* DNA when tested by PCR (Solano-Gallego et al., 2001). Of 73 clinically healthy hunting dogs in Greece, 12.3% tested positive at serology, whereas 63% were positive by PCR (Leontides et al., 2002). Longitudinal studies in endemic areas have shown that the natural history of the infected dogs can evolve in different ways (Oliva et al., 2006; Quinnell et al., 2001a). Two major patterns of progression have been documented. In some dogs, severe clinical signs of the disease appear shortly after infection. It is thought that animals with severe disease are unable to develop an effective cellular immune response, although they have a strong but ineffective humoral response (Pinelli et al., 1994; Barbieri, 2006). A second group of dogs remains infected for a long period of time (years or lifelong),

but they are able to avoid the appearance of lesions and clinical disease (Killick-Kendrick et al., 1994; Pinelli et al., 1994).

Transmission of *L. infantum* to vectors may occur from both asymptomtic and clinically ill dogs (Michalsky et al., 2007). However, infectiousness appears to be greater in clinically ill dogs (Guarga et al., 2000; Molina et al., 1999). In Figure 14, a lower infectiousness is represented by the dashed arrow connecting asymptomatic dogs and vectors. Nevertheless, since asymptomatic dogs are the majority of those that are infectious, they play a key role in transmission.

It must be remembered that both asymptomatic and clinically ill dogs account for prevalence of dogs positive to antibody detection (as evaluated in active data collection). Conversely, the transition from asymptomatic to clinically ill dogs affects incidence of clinical disease that is detected in passive data collection. Relationships between prevalence of antibodies and incidence of clinical leishmaniosis in dogs are analysed in a dynamic model described in Annex D.

8.1.2. Diagnosis of *L. infantum* in dog

Clinical signs in dog.

Canine visceral leishmaniosis is a multisystemic disease with variable clinical signs (Baneth, 2006; Ciaramella et al., 1997; Rallis et al., 2005). The majority of dogs are presented with poor body condition, generalized muscular atrophy, lymphadenomegaly, excessive skin scaling, dermatitis (Koutinas et al., 1999; Solano-Gallego et al., 2004; Papadogiannakis et al., 2005).

Renal disease might be the only apparent abnormality in infected dogs (Koutinas et al., 1999; Agut et al., 2003; Costa et al., 2003). A study on skeletal lesions in CanL found that out of 58 dogs with CanL, 45% had gait abnormalities. Ocular lesions are present in 16% to 80.5% of dogs with symptomatic CanL (Ciaramella et al., 1997; Koutinas et al., 1999; Peña et al., 2000; Naranjo et al., 2005). Nose bleeding (epistaxis), hematuria and hemorrhagic diarrhoea in CanL are associated with tissue ulceration and alterations in primary and secondary hemostasis. Anemia is present in the majority of symptomatic dogs because of chronic renal disease or decreased erythropoiesis caused by chronic disease, and can be aggravated by blood loss or immunemediated destruction of red blood cells (Ciaramella et al., 1997; Koutinas et al., 1999; Baneth et al., 2008). A comprehensive review of diagnostic criteria can be found in Paltrinieri et al (2010).

Laboratory diagnosis of L. infantum in dogs

Direct laboratory diagnosis

Until a few years ago the definitive diagnosis of CanL depended upon the demonstration of *Leishmania* spp. amastigotes in bone marrow or biopsy material (spleen, lymph nodes, liver). These parasitological tests are invasive and labour intensive, and thus are acceptable only in case of clinical suspicion of the disease. In addition, this technique has poor sensitivity so it cannot be considered a gold standard for CanL diagnosis (Gradoni, 1999; Ferroglio et al., 2007). Microscopy of stained smears from enlarged lymph nodes or from other damaged tissues can be nevertheless used in passive data collection.

In the last decade many PCR protocols to detect *L. infantum* DNA have been developed for the direct diagnosis of CanL, and PCR has been shown to be a sensitive and highly specific technique for the detection of infection. However, PCR is neither routinely used in clinical medicine nor in wide field surveys in many countries where the infection is endemic (Ashford et al., 1995, Quinnell et al., 2001a; Lachaud et al., 2002; Reithinger et al., 2002). Nested PCR and real time PCR are available.

Indirect laboratory diagnosis

Serologic methods are frequently used for mass screening of infected dogs and Immunofluorescent Antibody Test (IFAT) is widely diffused for diagnosis. Although IFAT represents the reference test, it is limited by the subjective interpretation of results, often non repeatable from different laboratories

(Reed, 1996). IFAT response is considered unequivocal for serum titres <1:40 (negative) or ≥1:160 (positive), but it is ambiguous for titres of 1:80 and 1:40 which are evaluated as doubtful (Ferroglio et al., 2002).

Recently, Western blot (WB) has proved to be more sensitive than IFAT and many authors have suggested its use in the diagnosis of CanL (Aisa et al., 1998; Fernández-Pérez et al., 1999). Unfortunately WB is labor intensive and it cannot be suggested for routine diagnosis (Ferroglio et al., 2007).

Enzyme linked immunosorbent assay (ELISA) and direct agglutination tests have been developed and are available for diagnostic laboratories (Scalone et al., 2002; Boarino et al., 2005). ELISA is a candidate for the development of a rapid and reliable *Leishmania* diagnostic method because it is more practical, standardisable and suitable for mass screening than IFAT. Specificity and sensitivity of the ELISA based immunoassay strictly depends on antigen quality and can be improved by the use of recombinant technology, which drives the expression, and purification of diagnostically relevant proteins in large amount (Reed, 1996; Sundar and Rai, 2002; Badaró et al., 1996; Burns et al., 1993; Zijlstra et al., 1998; Braz et al., 2002; Altintas et al., 1998; Ibrahim et al., 1999; Zerpa et al., 2000; Ozensoy et al., 1998; Kumar et al., 2001; Qu et al., 1994; Singh et al., 1995; Houghton et al.,1998; Gradoni and Gramiccia, 2000, Rosati et al., 2003; Boarino et al., 2005).

Rapid tests, using even whole blood samples, are now available, and producing companies declare 99.2% specificity and 96.3% sensitivity (Ferroglio et al. 2007). However, results of comparative studies are affected by the tests that are used as gold standard and, therefore, conflicting results are obtained. In fact, in a review by Paltrinieri et al (2010) certain rapid immunochromatographic tests were attributed poor sensitivity in comparison with IFAT and ELISA.

8.1.3. Data collection on *L. infantum* in animals by competent authorities of EU countries, Norway and Switzerland

Summary of results of questionnaires to the Task Force on zoonoses data collection

Eleven MSs, Denmark, Estonia, Finland, France, Hungary, Latvia, Portugal, Romania, Slovakia, Slovenia, Sweden, and Norway and Switzerland responded to the questionnaires regarding *L. infantum.*

L. infantum is notifiable in animals in Estonia, Norway, Romania, Slovenia, and Sweden.

Both active and passive data collections are carried out in France and in Sweden.

Only passive data collection is carried out in Portugal, Slovakia, and Slovenia.

In Denmark, Estonia, Finland, Hungary, Latvia, Norway, Romania, and Switzerland no data collection activity (active/passive) in animals is carried out.

Country by country results

Country by country results are reported in Annex A. Passive and active data collection activities on *L. infantum* are summarised in Table 37.

Table 37. Summary of results of the questionnaire on active and passive data collection on *L. infantum* in animals.

Country	Active data collection			Passive surveillance			National Reference Laboratory
	Time span	*Animal species*	*Diagnosis*	*Time span*	*Animal species*	*Diagnosis*	
Estonia							Estonian Veterinary and Food Laboratory
France		Dog	PCR, protein electrophoresis		Dog	PCR, protein electrophoresis	Centre National de Référence des Leishmania at CNRS-IRD, Montpellier University
Hungary							Central Agricultural Office
Portugal					Dog		Laboratório Nacional de Investigação Veterinária
Romania							National Reference Laboratory for vectors of animal diseases and zoonoses
Slovakia				2005 – 2009	Dog	Indirect ELISA	State Veterinary and Food Institutes
Slovenia					Dog	Indirect IFAT	Veterinary Administration of Republic of Slovenia
Sweden		Dog	Indirect ELISA		Dog	Indirect ELISA	National Veterinary Institute

[(a)] No data collection activities nor reference laboratory were reported in the questionnaires from Denmark, Finland, Latvia, Norway, Switzerland.

8.1.4. Systematic literature review of data collection on *L. infantum* in animals

The literature search was carried out on publications from 1990 through 2010. Results by country and information on diagnostic tests and sampling are presented in Annex B.

Results of active data collection on L. infantum by animal species

Main results of active data collection are summaries in Table 38. Data from seventy one publications were used in prevalence calculation, from 10 countries: Cyprus, France, Germany, Greece, Italy, Malta, Netherlands, Portugal, Spain, and Switzerland.

Interestingly, relatively high levels of prevalence were reported in wild animals, confirming susceptibility of these species to *L. infantum*, and suggesting that their role in transmission is worth being investigated. Due to large number of tested dogs, prevalence was estimated with good precision. Such an estimate was, however, affected by intensity of transmission in areas where data collection was carried out and should not, therefore, be interpreted as representative at the European level. Prevalence in other domestic animal species is also noteworthy. Antibodies against *L. infantum* were also detected in birds (geese and pheasants) (see Annex B for further details).

Table 38. Results of active data collection on *L. infantum* in animal species, based on the systematic review of publications in the EU, Norway and Switzerland from 1990-2010(a).

Animal species	N of countries	N of collection activities	N of tested animals	N of positive animals	Prevalence %	Lower 95% CL	Upper 95% CL
Dog[(b)]	10	67	66766	10640	15.94	10.25	23.94
Cat[(c)]	4	10	2112	231	10.94	4.20	25.60
Fox[(d)]	2	5	396	114	28.79	12.02	54.47
Wolf[(e)]	2	3	42	11	26.19	13.56	44.53
Horse[(f)]	2	2	125	17	13.60	11.37	16.19
Other carnivores[(g)]	1	4	16	4	25.00	20.34	30.32
Rats[(h)]	1	2	494	36	7.29	3.77	13.61
Sheep[(i)]	1	1	151	21	13.91	9.25	20.39
Goats[(j)]	1	1	147	15	10.20	6.25	16.24
Birds[(k)]	1	1	121	4	3.31	1.25	8.48
Small mammals[(l)]	1	1	121	0	0.00	0.00	0.03

(a) Prevalence estimates cannot, be considered as representative for each animal species since the results need to be interpreted after taking into account specific objectives, sampling design, and diagnostic tests used in each study; (b) Abranches et al., 1991, Alonso et al., 2010, Amela et al., 1995, Amusategui et al., 2004, Aoun et al., 2009, Baldelli et al., 1992, Baldelli et al., 2001, Brandonisio et al., 1992, Cabezón et al., 2010, Capelli et al., 2003, Cardoso et al., 2004a, Cardoso et al., 2007, Cardoso et al., 2004b, Cortes et al., 2007, Couto et al., 2010, Cringoli et al., 2002, Deplazes et al., 1995, Deplazes et al., 1998, Dereure et al., 2009, Federico et al., 1991, Fernández-Bellon et al., 2008, Ferroglio et al., 2005, Fisa et al., 1999, Gálvez et al., 2010, Headington et al., 2002, Leontides et al., 2002, Maia et al., 2010, Maresca et al., 2009, Maroli et al., 2001, Maroli et al., 2008, Martín-Sánchez et al., 2009, Mazeris et al., 2010, Menn et al., 2010, Miró et al., 2007, Mollicone et al., 2003, Morosetti et al., 2009, Neogy et al., 1992, Orndorff et al., 2000, Otranto et al., 2007, Papadopoulou et al., 2005, Paradies et al., 2006, Paradies et al., 2007, Rossi et al., 2008, Sánchez et al., 1996, Semião-Santos et al., 1995, Solano-Gallego et al., 2001, Tabar et al., 2008, Tabar et al., 2009, Teske et al., 2002, Zaffaroni et al., 1999, Keck et al., 2003, Nieto et al., 1992, Poglayen et al., 1997; (c) Ayllon et al., 2008, Diakou et al., 2009, Duarte et al., 2010, Maia et al., 2008, Maia et al., 2010, Martín-Sánchez et al., 2007, Poli et al., 2002, Solano-Gallego et al., 2007, Vita et al., 2005, Cardoso et al., 2010; (d) Criado-Fornelio et al., 2000, Dipineto et al., 2007, Fisa et al., 1999, Mancianti et al., 1994, Sobrino et al., 2008; (e) Sastre et al., 2008, Sobrino et al., 2008; (f) Fernández-Bellon et al., 2006, Rolão et al., 2005; (g) Sobrino et al., 2008; (h) Psaroulaki et al., 2010; (i) Fisa et al., 1999; (j) Fisa et al., 1999; (k) Otranto et al., 2010; (l) Fisa et al., 1999.

Results of passive data collection on L.infantum in animals

Results from passive data collection are shown in Table 39. In addition to dogs, cats and horses were also found positive for *L. infantum*. See Annex B for details on the criteria for suspect cases and diagnostic tests for confirmation.

Table 39. Results of passive data collection on *L. infantum* in animal species, based on the systematic review of publications in the EU, Norway and Switzerland from 1990-2010.

Species	Country	N of collection activities	N of suspected cases	N of confirmed cases
Dog[(a)]	Greece	3	1246	613
Dog[(b)]	Netherlands	2	598	146
Dog[(c)]	United Kingdom	2	258	258
Dog[(d)]	Portugal	3	102	36
Dog[(e)]	Spain	2	73	73
Dog[(f)]	Italy	2	16	16
Dog[(g)]	Poland	1	1	1
Horse[(h)]	Spain	1	3	3
Horse[(i)]	Germany	1	1	1
Cat[(j)]	Switzerland	2	2	2
Cat[(k)]	France	1	1	1
Cat[(l)]	Italy	1	1	1
Cat[(m)]	Portugal	1	1	1

(a) Mylonakis et al., 2008, Saridomichelakis et al., 2007, Sideris et al., 1996 ; (b) Teske et al., 2002, Wolschrijn et al., 1996; (c) Shaw et al., 2008, Shaw et al., 2009; (d) Abranches et al., 1991, Cardoso et al., 2002, Santos et al., 2006; (e) Martín-Sánchez et al., 1999, Todoli et al., 2009; (f) Foglia Manzillo et al., 2006, Tarantino et al., 2001; (g) Sapierzyński et al., 2008; (h) Solano-Gallego et al., 2003; (i) Koehler et al., 2002; (j) Rüfenacht et al., 2005; (k) Ozon et al., 1998; (l) Poli et al., 2002; (m) Marcos et al., 2009.

8.1.5. Information on the occurrence and geographical distribution of *L. infantum*, vectors, host.

Agent

Distribution, up to 2009, of agents causing human leishmaniasis, including *L. infantum*, and vectors, can be found in Ready (2010).

Based on our systematic literature review of publication from 1990 through 2010, animals positive for *L. infantum* were found in most of the countries where active data collection was carried out, with the exception of Switzerland, where 371 dogs were negative (Deplazes et al., 1995) (Table 40). In The Netherlands, only one out of 1971 dogs was positive (Teske et al., 2002). Most of the dogs that were examined in Germany, by active data collection, were either imported from, or traveled to Southern European countries, whereas no information was available for a small part of the sample (Menn et al., 2010).

By passive data collection, a horse was confirmed as infected by *L. infantum* in Spain (Solano-Gallego et al., 2003), and an autochthonous case of cutaneous leishmaniosis in another horse in Germany cannot be excluded (Koehler et al., 2002) (Table 39). *L. infantum* positive dogs that were found in The Netherlands and in The United Kingdom, by passive data collection, had a history of travel abroad, or no information was available in certain cases (Teske et al., 2002; Shaw et al., 2008, Shaw et al., 2009). Two imported cats were *L. infantum* positive in Switzerland (Table 39).

Table 40. Geographic distribution of *L. infantum* in animals in European countries, based on systematic literature review of active data collection from animals, from 1990 through 2010[(a)].

Country	N of animal species	N of studies	N of tested animals	N of positive animals	Prevalence %	Lower 95% CL	Upper 95% CL
Spain[(b)]	9	31	10645	1279	12.02	8.88	16.06
Italy[(c)]	4	33	36662	6996	19.08	11.98	29.00
Portugal[(d)]	4	15	8838	919	10.40	5.39	19.13
Cyprus[(e)]	2	5	4352	612	14.06	8.19	23.09
Greece[(f)]	2	3	1907	509	26.69	17.89	37.83
France[(g)]	1	6	1064	166	15.60	7.18	30.63
Germany[(h)]	1	1	4681	569	12.16	11.25	13.12
Netherlands[(i)]	1	1	1911	1	0.05	0.01	0.37
Switzerland[(j)]	1	1	371	0	0.00	0.00	0.01
Malta[(k)]	1	1	60	42	70.00	57.33	80.21

(a) Prevalence estimates cannot be considered as representative for each country since the results need to be interpreted after taking into account tested animal species, specific objectives, sampling design, and diagnostic tests used in each study, further details by country can be found in Annex B; (b) Alonso et al., 2010, Amela et al., 1995, Amusategui et al., 2004, Ayllon et al., 2008, Cabezón et al., 2010, Couto et al., 2010, Criado-Fornelio et al., 2000, Fernández-Bellon et al., 2006, Fernández-Bellon et al., 2008, Fisa et al., 1999, Gálvez et al., 2010, Martín-Sánchez et al., 2007, Martín-Sánchez et al., 2009, Miró et al., 2007, Sánchez et al., 1996, Sastre et al., 2008, Sobrino et al., 2008, Solano-Gallego et al., 2001, Solano-Gallego et al., 2007, Tabar et al., 2008, Tabar et al., 2009, Nieto et al., 1992; (c) Baldelli et al., 1992, Baldelli et al., 2001, Brandonisio et al., 1992, Capelli et al., 2003, Cringoli et al., 2002, Dipineto et al., 2007, Federico et al., 1991, Ferroglio et al., 2005, Mancianti et al., 1994, Maresca et al., 2009, Maroli et al., 2001, Maroli et al., 2008, Mollicone et al., 2003, Morosetti et al., 2009, Orndorff et al., 2000, Otranto et al., 2007, Otranto et al., 2010, Paradies et al., 2006, Paradies et al., 2007, Poli et al., 2002, Rossi et al., 2008, Vita et al., 2005, Zaffaroni et al., 1999, Poglayen et al., 1997; (d) Abranches et al., 1991, Cardoso et al., 2004a, Cardoso et al., 2007, Cardoso et al., 2004b, Cortes et al., 2007, Duarte et al., 2010, Maia et al., 2008, Maia et al., 2010, Menn et al., 2010, Rolão et al., 2005, Sastre et al., 2008, Semião-Santos et al., 1995, Cardoso et al., 2010; (e) Deplazes et al., 1998, Mazeris et al., 2010, Psaroulaki et al., 2010; (f) Diakou et al., 2009, Leontides et al., 2002, Papadopoulou et al., 2005; (g) Aoun et al., 2009, Dereure et al., 2009, Neogy et al., 1992, Keck et al., 2003; (h) Menn et al., 2010; (i) Teske et al., 2002; (j) Deplazes et al., 1995; (k) Headington et al., 2002.

Hosts

The distribution of canine population was not found in official sources of data such as Eurostat.

8.2. Proposal for data collection on *L. infantum* in animals

Dogs are main reservoir hosts and sources of *L. infantum* in Europe and, therefore, data collection is mostly targeted to this animal species. On the other hand, as previously underlined, dynamics of infection of dogs, pathogenesis, and immune response are complex. As a consequence, when planning data collection, and in the interpretation of results, such a complexity needs to be taken into account. Based on previous knowledge, and on a dynamical model of *L. infantum* in dog populations (Annex D), we can draw the following conclusions that should be taken into account in data collection.

The response of prevalence of antibodies against *L. infantum* in dogs to increasing trends of infected vectors is slow in an intial phase of invasion of new areas (previously free from *L. infantum*), while it is relatively rapid in a subsequent phase.

The incidence of clinical cases of infection in dogs shows the same trend as the prevalence of antibodies, since clinically ill dogs originate from asymptomatic dogs that are the majority of serologically positive dogs.

Since only a fraction of asymptomatic dogs develop clinical signs, the detection of clinically ill dogs, in areas favourable to sand fly vectors, is accompanied by the presence of greatest numbers asymptomatic dogs. Specifications for data collection on *L. infantum* in dogs are described below, and summarised in Table 41.

8.2.1. Data collection on *L. infantum* in dog

Specifications for active data collection of L. infantum in dog
Subpopulations to be covered

Dogs living below 800 m above the sea level, kept outdoor overnight or where vectors can access. Dogs treated with repellents (including collars) can be included provided that information on repellent is recorded and reported. Dogs can be clinically ill as long as this does not affect the probability of sampling (clinically ill dogs should have the same probability of sampling as other dogs).

Sampling context and design, sampling stage

Dogs can be sampled randomly through anagraphic lists or, more realistically, through convenience sampling based on owner compliance. Dog sera routinely collected in certain areas, for testing for *L. infantum* or other agents can be included in data collection as long as information is available on dog housing and treatments. Stratification at the second order administrative level, and reporting at the first level would improve representativity of samples.

Type of specimen to be taken and sampling technique

Blood serum.

Diagnostic methods to be used

ELISA tests are proposed in active data collection, considering standardised execution and objective interpretation of results. ELISA tests are generally accurate, especially those using recombinant antigens.

Data submission

In addition to number of tested and positive dogs, supplemental information on administrative area, housing, treatment, clinical status should be provided.

Specifications for passive data collection of L. infantum in dog
Subpopulations to be covered

Criteria for definition of suspect cases include clinical signs described in section 8.1. Moreover, see Paltrinieri et al. (2010) for guidelines for diagnosis. In summary, *L. infantum* should be suspected in dogs showing weight loss, cutaneous changes, lymph node enlargement, polyuria-polydipsia, epistaxis.

Type of specimen to be taken and sampling technique

Blood serum, biopsy from enlarged lymph nodes or from bone marrow, skin, conjunctiva, buffy coat, and whole peripherally obtained blood.

Diagnostic methods to be used

ELISA using recombinant antigens, for antibody detection. Note that, in spite of good specificity of ELISA, predictive value of positive results (the probability that the subject is really infected by *L. infantum*, following a positive test result) is affected by the known frequency of *L. infantum* in a geographic area. As an example, in areas where *L. infantum* is only sporadically reported or it is absent, the predictive value of positive serological tests should be considered as not perfect in the absence of history of travel. As a consequence, confirmatory direct diagnosis (i.e. microscopy of

stained smears from enlarged lymph nodes or from other damaged tissues, PCR) and follow up of case, with subsequent repeated testing, should be carried out.

Data submission

Information on type of diagnostic test that was used should be reported

Risk based data collection strategies.

Given the heterogeneous geographic distribution of *L. infantum* and of vectors, different data collection strategies should be adopted after classification of areas based on epidemiological patterns of *L. infantum.*

Endemic areas

Definition: areas with endemic foci, where the infection is stable. Vectors are present, and infection prevalence in dogs around 5% and greater.

Proposed data collection: active data collection on dogs from areas < 800 m above the sea level. The objective is the detection of trends in prevalence in dogs, as an indicator of trends in entomological risk.

Areas with unstable outbreaks

Definition: areas previously disease-free where the length of vector activity season is long enough to allow vector population to be maintained, but too short to maintain the *Leishmania* circulation in perspective. Autochtonous *Leishmania* cases in dogs have been sporadically detected.

Proposed data collection: active data collection on dogs from areas < 800 m above the sea level. The objective is the early detection of new foci of the agent and to study their evolution in case of occurrence.

Areas at risk of introduction

Definition: areas of vector presence where the length of vector activity season is long enough to allow vector population maintenance, but too short to maintain the *Leishmania* circulation in perspective. Autochthonous *Leishmania* cases have never been reported but unstable outbreaks following to the importation of diseased dogs from endemic areas are possible.

Proposed data collection: passive data collection of clinical cases in dogs, with a strong information campaign that is necessary to improve the collaboration in data collection and the overall data collection sensitivity. The objective is detecting in a cost-effective way the disease introduction.

Vector- free areas

Definition: areas free from the vectors, and where introduction is considered unlikely due to unfavourable conditions.

Proposed data collection: passive data collection of clinical cases in dogs with minor investment in communication. The objective is the detection of expansion of the geographic range of *L. infantum*.

Table 41. Specifications for active and passive data collection on *L. infantum* in dogs.

Type of data collection	Subpopulations to be covered	Sampling context, stage	Sampling design	Type of specimes	Diagnosis
Active	Dogs living below 800 m on the seas level, kept outdoor overnight, or where vectors can access	Owner, by private veterinarians	Convenience or random sampling based on lists. Stratified by administative area	Blood serum	Indirect ELISA using recombinant antigens
Passive	All dogs	Criteria for suspect cases: weight loss, cutaneous changes, lymph node enlargement, polyuria-polydipsia, epistaxis	Reporting: stratified by administative area	Blood serum, whole blood, biopsy from organs (see text)	ELISA and confirmatory microscopic examination, PCR

8.3. References on *L. infantum*

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CONCLUSIONS

- Animals play a central role in the transmission cycles of agents of vector borne zoonoses by acting as reservoirs or amplifying hosts. Alternatively, animals can be classified as dead-end hosts when they are susceptible to infection, but they are not essential for the agents' maintenance and transmission. The role of animal species not only varies for different agents, but also varies for different subtypes of the same agent (such as, for example, B. burgdorferi sl genospecies, Leptospira servovars, Hantavirus subtypes).
- Diagnosis of infection by agents of vector-borne zoonoses in animals is based on laboratory techniques. In fact, when clinical signs are present, they alone are not sufficient to achieve a diagnosis. A direct laboratory diagnosis mostly relies upon PCR techniques that generally allow subtype characterization, but are technically complex and time consuming. On the other hand, tests for an indirect laboratory diagnosis are available, and rapid and accurate ELISAs have recently been developed for several agents, including B. burgdorferi sl. Unfortunately, estimates of sensitivity and specificity of laboratory tests are rarely available.
- Data collection by competent authorities of the countries that responded to questionnaires on agents of vector-borne zoonoses in animals is most frequently carried out on certain agents, including WNV that is a recently emerging threat to both human and animal health, and on Leptospira and F. tularensis, that have long been known as causes of well defined disease.
- Active data collection by competent authorities on B. burgdorferi sl is infrequent. This is surprising since B. burgdorferi sl includes the agents of Lyme borreliosis which is the most common vector borne zoonosis in temperate climates. Furthermore, the geographic range of these spirochetes is expanding across Europe.
- The implementation of passive data collection on B. burgdorferi sl in animals, on the other hand, raises some concern as to the validity of results, given the variability of clinical signs and the imperfect specificity of IFA which is used as a confirmatory test.
- In the systematic literature review on data collection on agents of vector borne zoonoses in animals, publications from research were mostly retrieved. These included a detailed description of methods used in all phases of data collection and were based on up- to- date laboratory techniques (especially the most recent ones).
- Several animal species were the objective of data collection for multiple agents and in relatively numerous countries. These included not only domestic animals such as dogs (that were tested in 10 countries for B. burgdorferi sl) and cattle (with > 20000 individuals that were tested for Leptospira in nine countries), but also wild animals. Large numbers of small mammals were tested for different agents in spite of difficulties in capture and handling in comparison with domestic animals. As an example, 12000 wild mice of a single species, Myodes glareolus, were tested for Hantavirus in 14 countries.
- In publications from research, data collection was affected by variable objectives, and by heterogeneous sampling designs and diagnostic methods. Calculated prevalence of agents by animal species cannot, therefore, be considered as valid estimates at the level of single countries or groups of countries. Therefore, a valid comparison of prevalence of agents among animal species and countries was not possible.
- The critical analysis of results from the systematic literature review was, however, useful in generating proposals for data collection. Data collections from many individuals in multiple countries provided the most useful information on prevalence of agents and subtypes. On the other hand, even data collections carried out at single locations provided information on real scenarios that could be used as guides for future data collections. As an example, the relatively

high prevalence of antibodies against F. tularensis that were found in horses and in goats in Italy, at locations where outbreaks of the infection in humans had occurred, suggests that data collection from livestock might be used to detect the circulation of the agent.

- Proposals for data collection included animal species, such as dogs, sharing habitats and lifestyle with people, which can, therefore, be used as indicators of human exposure to agents of vector borne zoonoses. The owner compliance is important to obtain representative samples and gathering reliable information on companion animals (such as habitat frequentation, travel, and treatments) for a valid interpretation of collected data.
- On the other hand, farm animals have several advantages since their health status is continuously monitored through observation of clinical signs on farms and pathological lesions at slaughter. Moreover, blood serum, which might be collected for different reasons, may be available for testing for antibodies against agents of vector borne zoonoses. The inclusion of farm animals in data collection is, however, conditional upon husbandry types including free ranging and exposure to vector borne agents. Furthermore, certain grazing areas may not be suitable for important vectors, such as I. ricinus (that is limited to woods in most European countries), and may not be representative of the habitats that are most often frequented by humans.
- Wild animals are less available for data collection in comparison with domestic species, and data collection must take place during hunting or trapping. Nevertheless, given their important role as indicators of environmental sources of vector borne agents (either as reservoirs, or dead end hosts), wild animals are proposed for data collection for several agents. As examples, transmission intensity of WNV can most effectively be evaluated by data collection from wild birds, and wild rodents are the most effective sources of data on TBEV given the generally low prevalence of this agent in tick vectors.
- Synanthropic animals are wild animals living at, or near human settings, including several bird species or groups (i.e. Corvidae) and rats and house mice among mammals. Their potential role as sources of vector borne agents for humans and the existence of population control plans make these species useful in data collection.
- Active data collection is recommended for all agents, whereas passive data collection is limited to animals suffering clinical consequences from infections.
- Proposed sampling contexts and stages for specimen collection (for example, blood) include the examination of companion animals by veterinarians, sampling of farm animals for different purposes, and meat inspection of carcasses by official veterinarians at slaughterhouse (for example, for the detection of lesions by Leptospira in pig kidneys), hunting and trapping.
- Stratification of sampling at least at the level of the first administrative divisions within each country is proposed to increase precision of prevalence estimates. Information on animal populations in each administrative area is required to allow adjustment for disproportionate stratified sampling (EFSA, 2009).
- Dynamic mathematical models were used, for a subset of agents, to point out essential transmission features and to explore the relationship between data collected on animals (i.e. prevalence of the agents, or incidence of clinical cases) and the intensity of transmission of the agents in the source of infection for animals and man (i.e. vectors or reservoir hosts). Key parameters (and gaps in knowledge) were identified, including contact rates between infection sources and animals, antibody response and decay, incidence of clinical disease.

- Models were used for sample size determination to identify epidemiologically meaningful trends in the prevalence of antibodies against agents of vector borne zoonoses, following increasing trends in the abundance of infected vectors.

RECOMMENDATIONS

- Data collection on vector borne agents in animals should be improved within the framework of Directive 2003/99/EC, because of the epidemiological situation characterised by increasing frequency and expansion of the geographic range of these agents.
- Different options for data collection (based on agents, animal species, and sampling specifications) should be carefully evaluated in individual countries, but also in the EU. In fact, in the case of vector borne zoonoses, spatial diffusion of agents across countries is as important as temporal trends within the same countries, therefore harmonization of data collection in different countries is recommended.
- The use of the same animal species for data collection on several microbial agents is recommended.
- Existing data collection activities on animals should be carefully considered as potential sources of information and of specimens on the agents included in this report.
- The complex ecological interactions among animal hosts, microbial agents, and arthropod vectors need to be considered both in planning the collection of data on agents of vector borne zoonoses in animals and also in the interpretation of results.
- When reporting results of data collection, the following information should be reported: dimension of animal target population, sampling design, sample size, primary sampling unit (individual animal, herd) information on sampling locations (administrative area, geographic coordinates), diagnostic test used, cut off value (if appropriate), number of positive individuals.
- Statistical analysis of data on agents of vector borne zoonoses should be carried out taking into account sampling design, including seasonal variations and spatial heterogeneity characterising the distribution of these agents (EFSA, 200913; EFSA 201114).
- The integration of information on agents of vector borne zoonoses from different sources, including animals, vectors, and humans, is recommended. The objective is to achieve a more effective evaluation of trends of these emerging agents. In fact, transmission may only occur where both animal hosts and vectors are present, and the risk for public health is largely affected by human behaviour (i.e. habitat frequentation, lifestyle).

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