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TITLE

Characterization of Respiratory Tract Microbiome in Healthy and Bovine Respiratory Disease (BRD) Affected Piedmontese Calves

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XXX Cycle

Summary

ABBREVIATIONS

ADD = Animal Daily Dose

APP = Acute Phase Proteins

ASAZ = *Associazione Servizi Agricoli e Zootecnici* (Association for Zootechnical and Agricultural Service)

ATC-Vet = Anatomical Therapeutic Chemical Classification for Veterinary medical products

BHV – 1 = Bovine Herpesvirus type 1

bp = basepairs

BRD = Bovine Respiratory Disease

BRSV = Bovine Respiratory Syncytial Virus

BVDV = Bovine Viral Diarrhea Virus

CALA = Computer-Aided Lung Auscultation

CI = Confidence Interval

CN = Cuneo

CRSC = Calf Respiratory Scoring Chart

CT = Comet Tail artifact

DNA = DesoxyriboNucleic Acid

ELISA = Enzyme Linked ImmunoSorbent Essay

ECDC = European Centre for Disease Prevention and Control

EFSA = European Food and Safety Authority

EMA = European Medicines Agency

FDR = False Discovery Rate

Fib = Fibrinogen

HP = Haptoglobin

IU = International Unit

LA = Long-Acting

LD = Legislative Decree

MIC = Minimal Inhibitory Concentration

NGS = Next Generation Sequencing

NPP = Negative Predictive Value

NS = Nasal swabs

OR = Odds Ratio

OTU = Operational Taxonomic Unit

PCoA = Principal Coordinates Analysis

PCR = Polymerase Chain Reaction

PERMANOVA = Permutational Analysis of Variance

PI3 = Parainfluenza 3 Virus

PPV = Positive Predictive Value

QIIME = Quantitative Insights Into Microbial Ecology

RD = Recommended Dose

RNA = RiboNucleic Acid

ROM = Reactive Oxygen Metabolites

RR = Respiratory Rate

rRNA = ribosomial RiboNucleic Acid

RT – PCR = Reverse Transcriptase Polymerase Chain Reaction

SAA = Serum Amyloid A

Sd = Standard deviation

Se = Sensitivity

SEM = Standard Error of the Mean

Sp = Specificity

TO = Turin

TTA = Trans-Tracheal Aspiration

TU = Thoracic Ultrasonography

UDD = Used Daily Dose

VC = Vercelli

WHO = World Health Organization

INTRODUCTION

The definition of Bovine Respiratory Disease (BRD) includes all forms of bronchopneumonia where the pathogens gain access to the lung through the upper respiratory airways (Woolums, 2015a). BRD pathogenesis is multifactorial and involves several management and environmental factors, which predispose to the colonization of the respiratory tract by etiological agents (Panciera and Confer, 2010; Taylor et al., 2010). Consequently, its morbidity and mortality may vary among different production categories, countries and years, depending on the pathological agents involved and the predisposing factors at which the animals are subject. However, the most affected cattle categories are those of young animals (Edwards, 2010; Stokka, 2010; Pardon et al., 2012a; Woolums, 2015a). Viruses and bacteria are both involved in the development of the disease (Panciera and Confer, 2010). Over the course of BRD, viruses mainly contribute to the reduction of the local and systemic immune defense and the establishment of an environment favorable to bacterial colonization (Schreiber et al., 2000; Jones and Chowdhury, 2010; Ridpath, 2010; Saif, 2010; Woolums, 2015a), while bacteria induce the most severe clinical signs, increasing mortality (Griffin et al., 2010).

As previously mentioned, the etiology of BRD is considered to be multifactorial, because factors other than infectious agents are involved in its development. Events such as weaning, castration, transportation, overcrowding and mixing animals coming from different sources can trigger stress-related processes, that affect cortisol levels and increase the oxidative stress, reducing immunity defense and predisposing the colonization by pathogenic agents (Chirase et al., 2004; Taylor et al., 2010). Other management factors can reduce immune defenses, leading to BRD, such as bad or no colostrum administration, or improper or lack of vaccination plans (Edwards, 2010; Gorden and Plummer, 2010). Finally, factors related to the animals can contribute to the development of respiratory disease as well, such as age, sex, weight or breed type (Taylor et al., 2010).

BRD EPIDEMIOLOGY

Bovine Respiratory Disease is one of the primary causes of morbidity and mortality in calves, of both beef and dairy breed (Edwards, 2010; Stokka, 2010; Pardon et al., 2012a; Woolums, 2015a).

The prevalence of clinical BRD is variable, depending on infectious agents involved, the affected animal category and the management and environmental characteristics. As reported by a USA National Animal Health Monitoring System study of 2011, clinical BRD is the disease with the highest incidence (16.2%) and highest average treatment cost (23.6 $) in feedlot cattle (USDA, 2013). Earlier studies, conducted in USA feedlots, reported similar (17 %) or lower (8.17%) incidence (Snowder et al., 2006; Schneider et al., 2009). Another study conducted in Brazil, reported a lower incidence (6.13%) and a percentage due to BRD of 0.21%, but BRD was still the primary cause of morbidity and mortality (Baptista et al., 2017). In northwestern France the BRD incidence was 18.5 % in fattening operations (Assié et al., 2009). Moreover, the majority of the case of BRD are reported in the first two months on feedlot (Schneider et al., 2009). Regarding beef cow-calf production type, the BRD incidence in pre-weaned calves reflected those found in other production categories. Charolaise pre-weaned calves, from France, showed a BRD incidence of 15%; in Canada, the incidence risk for BRD was 3% in pre-weaned calves, while in USA the BRD average annual incidence was 10.5% (Assié et al., 2004; Snowder et al., 2005; Murray et al., 2016). Furthermore, In dairy calves the incidence of BRD varied from 5.7% to 7.6%, depending on the age of the animals, and it is considered as the most common cause of death (Sivula et al., 1996; Svensson et al., 2003, 2006a; b). Finally, in veal calf category, the incidence of clinical BRD was < 7% in Italy, France and Netherlands, while in Belgium the it was 14.8%, with a mortality rate of 1.3% (Brscic et al., 2012; Pardon et al., 2012a, 2013). Finally, in

Nevertheless, prevalence of BRD could be underestimated, if calculated only on the presence of clinical signs. In fact, studies evaluating post-mortem lung lesions, reported higher percentage of affected animals. For example, Schneider et al. (2009) reported a clinical BRD incidence in feedlot of the 8.17%, but the 61.9% of animals had lesions at slaughter. A recent Italian study reported a similar amount of animals with lung lesions at post-mortem examination (64%) (Caucci et al., 2018). Moreover, Leruste et al. (2012) obtained similar results on veal calves: the percentage of presence of clinical signs went from 0.7 to 6.8%, depending on the analyzed period and the considered sign, yet more than half of the animals had different degrees of lung lesions at post-mortem examination.

These data explained how BRD has a great economic impact on the cattle industry. In fact, economic losses can be attributed not only to medical treatment and animal death, but to a reduction of the productivity as well. This is represented by a reduction of the average daily gain (ADG) and of the carcass quality in beef and veal calves; and by a major risk of infertility and culling in dairy heifers (Schneider et al., 2009; Pardon et al., 2013; Teixeira et al., 2017).

BRD ETIOLOGICAL AGENTS

Viruses

Principal viruses associated with BRD are: Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza-3 virus (PI3), Bovine Herpesvirus-1 (BHV-1), Bovine Viral Diarrhea Virus (BVDV) and Bovine Coronavirus (BCoV) (Brodersen, 2010; Ellis, 2010; Jones and Chowdhury, 2010; Panciera and Confer, 2010; Saif, 2010). Most of these viruses have been primarily described as predisposing agents, rather than direct responsible of the appearance of clinical signs, by inducing the alteration of the respiratory mucosa and immune systems, thus predisposing to the colonization by bacterial agents (Lopez et al., 1976; Yates, 1982; Panciera and Confer, 2010). BRSV is the only virus that has been isolated alone in severe BRD cases with high fever, dyspnea, cough and nasal discharge, and it has been correlated with fatal cases in both young and adult cattle (Ellis et al., 1996; Schreiber et al., 2000; Brodersen, 2010; Woolums, 2015a). BRSV, together with PI3, belong to genera *Pneumovirus* and *Respirovirus*, respectively, both classified in the family Paramyxoviridae, and they are principally respiratory pathogens, transmitted by direct contact or aerosol (Woolums, 2015a). Contrarily to BRSV, PI3 induce sub-clinical or mild signs and its principal role in BRD is to predispose the respiratory tract to colonization by bacteria or other viruses (Lopez et al., 1976; Ellis, 2010; Woolums, 2015a). BHV-1 is a *Varicellovirus* belonging to family Herpesviridae, whose infection in cattle is correlated to BRD development in two ways. The BHV-1 subtype 1, more diffuse in feedlot cattle, is the etiological agent of bovine rinotracheitis, characterized by high fever, conjunctivitis and ocular discharge, nasal discharge, dyspnea and inflamed nares (Jones and Chowdhury, 2010). Moreover, it can transiently suppress the immune systems, hence predisposing infected animals to secondary bacterial infections (Yates, 1982; Jones and Chowdhury, 2010). The term BVDV refers to two species, BVDV 1 and BVDV 2, belonging to genus *Pestivirus*, family Flaviviridae (Ridpath, 2010). They can induce several different diseases, including subclinical infection, bovine viral diarrhea and mucosal disease, immunosuppression, abortion and fetal mummification, congenital defects and persistent infections (Walz, 2015). The primary role of BVDV in BRD development is correlated with immunosuppression, which predisposes to infections by other agents and have a synergic effect with them, leading to more serious clinical signs in BVDV positive animals than in BVDV negative animals (Fulton et al., 2000; Connor et al., 2001; Burciaga-Robles et al., 2010; Ridpath, 2010; Woolums, 2015a). Nevertheless, experimental infection with BVDV in 6-month old calves induced mild respiratory clinical signs (Potgieter et al., 1984). BCoV, belonging to family Coronaviridae, is correlated both with respiratory and enteric infections in cattle, and animals infected with BCoV may have both respiratory and enteric symptoms consequent to one infection (Cho et al., 2000). Experimental infections, however, showed that BCoV may induce lung damages, but cannot be considered the cause of death, since the respiratory infections that induces are mild (Kapil et al., 1991). Other rhinoviruses (Rhinoviridae) and adenoviruses (Adenoviridae) have been considered as minor pathogens in BRD development and a recent

metagenomic study highlighted their possible contribution (Ng et al., 2015). The authors found a higher prevalence of bovine adenovirus 3 and bovine rhinitis A virus in BRD affected animals, compared with healthy subjects (Ng et al., 2015). Finally, a new influenza virus, the influenza D virus, was identify by metagenomics characterization of virome and a possible role in BRD development of this virus was suggested (Ng et al., 2015; Mitra et al., 2016).

Bacteria

The main bacterial agents identify in BRD are: *Pasteurella multocida, Mannheimia haemolytica*, *Histophilus Somni* and *Mycoplasma bovis* (Griffin et al., 2010). The aforementioned species have been commonly identified in the upper respiratory tract of both healthy and unhealthy subject, indicating that they could be common inhabitants of the upper respiratory tract (Panciera and Confer, 2010). Following the impairment of general and local immune defense, they can colonize the lower respiratory tract and induce the disease (Panciera and Confer, 2010). *P. multocida, M. haemolytica* and *H. somni* are all Gram-negative aerobic bacteria, belonging to family *Pasteurellaceae* (Woolums, 2015a). Twelve serotypes of *M. haemolytica* species were reported, with serotypes A1 and A6 being the most common in lung from BRD-affected animals (Griffin et al., 2010; Klima et al., 2014a). *P. multocida* has 5 capsular serogroups and 16 somatic serotypes (Griffin et al., 2010). There lies a correlation between the serotypes and serogroups and the predisposition for the colonization of specific organs or species (Woolums, 2015a), and the most abundant serogroup which was mostly isolated in case of BRD is *P. multocida* A:3 (Dabo et al., 2008). Both *P. multocida* and *M. haemolytica* induced important clinical signs after experimental inoculation, albeit *M. haemolytica* apparently produced more severe clinical and pathological changes, likely dues to its rapid growth and the production of numerous virulence factors, the most important being a leukotoxine (Dowling et al., 2002; Panciera and Confer, 2010). Although the aforementioned species were frequently isolated in case of BRD, *P. multocida* seems to be more involved in the respiratory disease of neonatal calves, whereas *M. haemolytica* assumes a more important role in respiratory disease of post-weaned beef calves (Rice et al., 2007; Dabo et al., 2008; Klima et al., 2014b). Indeed, *P. multocida* is an important pathogen in BRD (Ames et al., 1985; Nikunen et al., 2007a), but it has been frequently isolated also in the upper and lower respiratory tract of healthy calves. In a study carried out in Scottish calves without clinical signs, 17% had bacterial culture of nasopharyngeal swabs positive for *P. multocida* (Hotchkiss et al., 2010). Furthermore, Taylor et al. (2015) found that *P. multocida* was more frequent in not-treated calves, and Francoz et al. (2015) did not find a correlation with the presence of this pathogen in upper respiratory tract and the manifestation of clinical signs. Concerning the lower respiratory tract, Allen et al. (1991) found similar frequency of isolation of *P. multocida* in both healthy and BRD affected calves, even if it was correlated with morbidity. Angen et al. (2009) isolated *P. multocida* more frequently in diseased calves, but the difference with healthy ones was not significant. *M. haemolytica* was most frequently associated with

the clinical disease (Booker et al., 2008; Timsit et al., 2013; Taylor et al., 2015). Taylor et al. (2015) isolated *M. haemolytica* more frequently from nasopharynx of treated animals, compared to control. However, this species has also been detected in samples of healthy animals. Angen et al. (2009), for instance, found no significant differences in *M. haemolytica* isolation in lower respiratory tract of calves with or without clinical signs (Angen et al., 2009), and this species has also been identified in the upper respiratory tract of healthy calves (Allen et al., 1991). It is worth noting that serotyping of the isolated bacteria has been rarely performed, making it difficult to define whether the isolated species belonged to a pathogenic serotype or not. For example, the serotype 2, , was primarily isolated in nasal swabs collected from healthy calves (Klima et al., 2014a). *H. somni* is recognized as an important component in BRD etiology, but it can produce other clinical syndromes, such as thrombotic meningoencephalitis (TME), polysynovitis and polyarthritis, septicemia, myocarditis and pericarditis, otitis media, infertility, abortion, and mastitis (Griffin et al., 2010; Headley et al., 2013). Though it was isolated both in upper and lower respiratory tract, this species seems to predilect lung colonization (Griffin et al., 2010; Doyle et al., 2017). It has also been isolated in the lower respiratory tract of calves with or without BRD clinical signs (Angen et al., 2009).

M. bovis belongs to class Mollicutes, a group of bacteria without cell walls, a feature that provides them natural resistance against beta-lactam antibiotics (Maunsell and Donovan, 2009; Caswell et al., 2010). *M. bovis* is an important pathogen in BRD, but it is also involved in development of otitis, polyarthritis and mastitis (Pfützner and Sachse, 1996; Arcangioli et al., 2008; Woolums, 2015a). In fact, its isolation in both upper and lower respiratory tract was frequently correlated with the disease. Francoz et al. (2015) found *M. bovis* as the sole bacterium isolated from nasal swabs significantly correlated with BRD. Moreover, it was frequently isolated from lungs of BRD affected animals in Belgium, Britain and France (Thomas et al., 2002a; Ayling et al., 2004; Arcangioli et al., 2008). However, it has also been identified in lung lower respiratory tract samples of healthy calves. Other species belonging to *Mycoplasma* genus has been isolated from upper and lower respiratory tract of calves, such as *M. dispar* or *M. bovirhinis* (Allen et al., 1991; Thomas et al., 2002a; Ayling et al., 2004; Angen et al., 2009). The former was frequently isolated from lung of BRD affected calves, its identification being less frequent than *M. bovis* (Allen et al., 1992; Thomas et al., 2002a; Ayling et al., 2004). On the other hand, *M. bovirhinis* has been isolated in both healthy and BRD affected animals and it has been considered mostly an opportunistic bacterium (Allen et al., 1992; Thomas et al., 2002a; Ayling et al., 2004).

Among secondary pathogens, it is possible to find species belonging to the *Pasteurellaceae* and *Mycoplasmataceae* families, and *Trueparella pyogenes* (Griffin et al., 2010). *Bibersteinia trehalosi* is a species belonging to *Pasteurellaceae* family which was once classified as biotype T of *P. haemolytica* (Panciera and Confer, 2010). *B. trehalosi* is primarily an ovine pathogen, which causes septicemia and severe pneumonia in sheep (Confer, 2009). Recently, the presence of *B. trehalosi* was associated with severe case of pneumonia in cattle, although an experimental inoculation of *B. trehalosi* in healthy calves did not increase lung involvement or severity of clinical signs, when compared to control group

(Hanthorn et al., 2014). Consequently, *B. trehalosi* is not considered a primary BRD pathogen (Confer, 2009; Hanthorn et al., 2014). *Ureaplasma diversum* belongs to the family *Mycoplasmataceae* and, albeit not recognized as a major BRD pathogen, it has been isolated in affected calves or pneumonic lung (Thomas et al., 2002a; Autio et al., 2007). *T. pyogenes* is a Gram-positive bacterium, more frequently associated with bovine abscess, but can be isolated also in lung (Confer, 2009). It is an inhabitant of nasopharynx and many other mucosal surfaces (Confer, 2009). However, it is not recognized as a primary pathogen for BRD, but a secondary invader of lung, causing a chronic abscessing pneumonia (Confer, 2009).

PREDISPOSING FACTORS FOR BRD DEVELOPMENT IN BEEF CATTLE FATTENING OPERATIONS

Several management and environmental factors have been associated with BRD development (Edwards, 2010; Taylor et al., 2010). They can impair the immune response, allowing viral and bacterial pathogens to colonize the lower respiratory tract, leading to disease (Taylor et al., 2010).

Beef calves are frequently moved soon after weaning, transported for relatively long distances and housed with older animals or animals from others sources (Taylor et al., 2010). Abrupt weaning has been proved to be a stressful event for calves (Haley et al., 2005). It also caused increased concentrations of acute-phase proteins, cortisol and increases in the neutrophil-lymphocyte ratio within 5 days post-weaning (Kim et al., 2011). Moreover, Lynch et al. (2010) showed that abrupt weaning caused neutrophilia and reduced lymphocyte count within 2 days, while values returned to baseline after 7 days, therefore suggesting a transitory reduction of immune function consequent to weaning. In addition, transportation showed to increase stress biomarkers, such as cortisol, catecholamines and acute phase proteins concentrations (Arthington et al., 2003; Odore et al., 2004). Moreover, an alteration of lymphocyte subsets was identified both in blood and bronchoalveolar fluid samples, following to transportation, concurring to a possible impairment in immune function (Ishizaki et al., 2005; Riondato et al., 2008). Transportation encompasses many individual factors that can increase BRD morbidity, the most important being still debated. Pinchak et al. (2004) and Hay et al. (2014) reported differences in risk of BRD development correlating with transportation duration. Ribble et al. (1995), contrarily, suggested that travel distance had no effect in BRD development, and Cole et al. (1988) found a higher incidence in animals transported for 12 hours when compared with those transported for 24 hours; sorting, loading and early transit were pointed out as primary causes of transportation-related stress. Marques et al. (2012) underlined the important effect of water and food deprivation on stress in long-distance transportation. Commingling of animals from different sources has been also reported as a predisposing factor for BRD development (Sanderson et al., 2008; Step et al., 2008; Taylor et al., 2010). When regrouping for the first time, steers showed an increase in cortisol concentrations, indicating the grouping of unfamiliar animals as a stress source (Gupta et al., 2005). Hay et al. (2014) reported that the effect of mixing is also influenced by the number of sources from which cattle are purchased and the interval intercurrent between mixing and induction. Indeed, mixing of different animals is not only a cause of stress, but increase for the exposure of naïve animals (Sanderson et al., 2008), in a moment in which other stressful factors induce an impairment in immune defense. Another factor associated with BRD development is weather (Taylor et al., 2010). BRD incidence was correlated with low temperature, large temperature variation during the day and maximum wind speed (Cusack et al., 2007; Cernicchiaro et al., 2012). Moreover, many authors reported an increase of BRD incidence in autumn or winter seasons (Andrews, 1976; Hay et al., 2017). Ribble et al. (1995) correlated the higher incidence of BRD in the second half of October, compared to September, not only considering the weather, but also due to an increase in animal transportation and sell and others management factors.

Others predisposing factors are associated with the animal, such as gender, weight or breed (Taylor et al., 2010). Female calves are reported by some authors to experience less BRD morbidity and mortality, when compared to male calves (Muggli-Cockett et al., 1992; Gallo and Berg, 1995; Snowder et al., 2006; Cusack et al., 2007). Contrarily, another study conducted in US reported beef heifers to have an increased risk of dying for respiratory disease, compared to beef steers, from 1997 to 1999 (Loneragan et al., 2001). However, the authors also reported an increase in light-weight heifers importation in the same period, suggesting that this could be correlated with the increased mortality (Loneragan et al., 2001). Sanderson et al. (2008) did not find a difference between heifers and steers but identified the presence of mixed gender groups as a risk factors for BRD development. Bulls castrated after shipping were reported to have a higher morbidity, compared to steers, castrated before shipping (Berry et al., 2001; Pinchak et al., 2004). Castration, is a stressful event, that leads to an increase in cortisol concentrations, which can impair immune defense, predisposing to BRD development (Fisher et al., 1997; Pinchak et al., 2004; Burdick et al., 2011). Calves with lighter weight have been frequently reported to be more prone to develop BRD (Bateman et al., 1990; Gummow and Mapham, 2000; Sanderson et al., 2008; Hay et al., 2017). Body weight can be used as an approximation of age, and young calves are less likely to be exposed to pathogens and to subsequently have a complete immunity (Loneragan et al., 2001; Sanderson et al., 2008). In fact, Townsed et al. (1989) reported that younger calves had a high probability to develop fever. However, difference cut-offs or weight category were used in analysis. Gummow and Mapham (2000) divided the animals on the base of mean value, and they found that animals processed at a weight lower than 245 kg were 1.4 times more likely to develop respiratory diseases than heavier calves. Sanderson et al. (2008) highlighted the fact that calves weighing more than 318 kg were less predisposed to BRD and they found a trend for mid-range weighted calves (250-318 kg) to have fewer episodes of BRD, compared with lighter calves. Moreover, Hay et al. (2017) formed 4 categories, with the lower cut-off at 400 kg, which probably indicated older animals. Nevertheless, not all the authors reported an influence of weight in development of BRD. Gardner et al. (1999) and Alexander et al. (1989) did not report any differences in weight among health categories. The influence of breed in BRD incidence is still debated. Heritability in developing BRD has been proved to be low (Heringstad et al.; Mccorquodale et al.; Snowder et al., 2005). However, some studies reported certain breeds to seem more predisposed in developing BRD. Hereford breed, for instance, has been reported to have a higher incidence of BRD, compared to other breed (Durham et al., 1991; Snowder et al., 2006; Hägglund et al., 2007). Muggli-Cockett et al. (1992) reported Pinzgauer to have a higher incidence, compared to Angus, Charolais, Limousin, Gelbvieh and Red Poll. Concerning Angus, only one study identified this breed as more predisposed to treatment (Hägglund et al., 2007). Also heterozygosity was investigated, with conflicting results, by the same authors (Snowder et al., 2005, 2006).

IDENTIFICATION OF BRD AFFECTED CALVES

One of the most important challenge in controlling the spread of BRD pathogens is the early identification of affected animals. The difference between the percentage of animals classified as ill during the production cycle, compared with those that had lesions at post-mortem examination showed, instead, the large diffusion of not recognized cases (White and Renter, 2009; Leruste et al., 2012; Timsit et al., 2016a).

Clinical examination

The identification of clinical cases is usually performed by distant visual inspection followed by clinical examination. Clinical signs of BRD could be non-specific, consisting in fever, anorexia, depression and lack of rumen filling; or more correlated with respiratory tract, like tachypnea, dyspnea, cough, nasal discharge and ocular discharge (Apley, 2006; Woolums, 2015a). Head tilt or auricular ptosis could be also included in BRD clinical signs, given the common correlation between auricular and respiratory disease in calves (Bertone et al., 2015).

However, some of these signs are not BRD-specific and their identification and interpretation is mostly performed by farmers or feedlot personnel, and it is subjective. This leads to low accuracy of clinical observation of animals, with a sensitivity spanning from 61.8% to 27%, depending on the studies considered, and which is probably influenced by the number of animals to be checked by a single pen checker (White and Renter, 2009; Timsit et al., 2016a). Moreover, cattle has a natural tendency to hide any sign of weakness in human presence, due to its nature of prey species (Wolfger et al., 2015). Therefore, in order to standardize clinical examination, clinical scores were developed. The DART method, for example, relies on the diagnosis of four clinical signs: depression, appetite loss, respiratory character change and temperature augmentation (Griffin et al., 2010). So far, it has been widely used in order to decide which animals had to be treated, but the examined signs are not specific nor standardized (Griffin et al., 2010). Another clinical score was developed at the University of Wisconsin for the evaluation of dairy calves health (Calf Respiratory Scoring Chart, CRSC) (McGuirk and Peek, 2014). This method takes into account five clinical signs: body temperature, cough, nasal discharge, ocular discharge and head position. Each of these signs obtains a score varying from 0 to 3, depending on its severity. However, considering ocular discharge and head position, only that who scores the highest is chosen. The final record given to each calf can stretch from 0 to 12, and treatment is recommended to subjects with a score ≥ 5, while those with a score of 4 shall be monitored. Finally, animals with a score ≤ 3 are considered healthy (McGuirk and Peek, 2014). The CRSC showed low sensitivity (Se) and specificity (Sp) when compared both with thoracic ultrasonography (Se: 55.4%; Sp: 58%) and when evaluated by means of a Bayesian latent-class model (Se: 62.4%; Sp: 74.1%) (Buczinski et al., 2014, 2015). Further study reported a cut-off of ≥ 7 to be more valuable, showing high specificity (Sp: 89%), but still low sensitivity (Se: 35%), for the identification of sick animals (Francoz et

al., 2015). Finally, slight to fair agreement was found when different trained observers used the CRSC on the same calves, thus highlighting the high risk of this method in increasing false negative and false positive cases (Buczinski et al., 2016a). Consequently, considering the importance of an early treatment and the reduction of the antimicrobial usage, further tools need to be added to clinical examination, in order to increase the diagnostic accuracy of BRD-affected animals.

Thoracic auscultation

Thoracic auscultation allows to evaluate the presence of increase bronchial sounds, abnormal lung sounds, such as wheezes and crackles, produced by airflow alteration, following the presence of lung lesions (Terra and Reynolds, 2015). The accuracy of thoracic auscultation has been rarely evaluated in bovine medicine. In human and ovine species, it showed low accuracy in diagnosing lung diseases (Lichtenstein et al., 2004; Scott et al., 2010). In dairy calves the obtained results varied according to the definition of abnormal lung sound. In fact, excluding the presence of increased bronchial sound as abnormal finding, thoracic auscultation had very low sensitivity (ranged from 0 to 16.7%, depending on the lung site considered), but a very high specificity (> 97%) (Buczinski et al., 2014). On the other hand, the interpretation of increased bronchial lung sound as abnormal finding increased the sensitivity (Se = 73%) of thoracic auscultation, but decreased the specificity (Sp = 53%) (Buczinski et al., 2016c). As a clinical examination, thoracic auscultation is subjective and it requires a specific training, in order to be able to differentiate normal and abnormal lung sounds (Mang et al., 2015). Moreover, abnormal lung sounds, as the presence of an increased bronchial sound, could also derive from other causes, different from respiratory disease (Buczinski et al., 2016c). In order to overcome these issues, a computer-aided lung auscultation (CALA) system has been developed. The CALA system showed high accuracy (Se: 93%; Sp: 90%) and substantial agreement with the lung auscultation performed by a trained veterinary ($\kappa = 0.77$) (Mang et al., 2015).

Thoracic ultrasonography

Thoracic ultrasonography proved to be an accurate and practical technique to diagnose lung disease in human, dogs, cats and foals (Lichtenstein et al., 2004; Ramirez et al., 2004; Ward et al., 2017). This technique is a non-invasive diagnostic tool that may be useful in bovine practice for the diagnosis of BRD, as it can be easily performed in the field, on a non-sedated, standing animal (Babkine and Blond, 2009). Linear or sectorial probes, with frequency ranges from 7.5 to 3.5 MHz, may be employed (Babkine and Blond, 2009). All intercostal spaces from the 11^{th} to the 2^{nd} are evaluated, in dorso-ventral direction (Ollivett and Buczinski, 2016). Considering that the cranio-ventral part of the lungs are the most affected in case of BRD, it is important to concentrate the examination on this parts (Panciera and Confer, 2010; Ollivett and Buczinski, 2016). Consequently, linear rectal probes are preferable, because

their shape allows better access to the cranio-ventral part of the thorax (Ollivett and Buczinski, 2016). In order to improve image quality, isopropyl alcohol should be used as the transducing agent, avoiding to trim or shave the hair from the chest (Ollivett and Buczinski, 2016). When evaluating a healthy lung, pleural line is the only finding that can be appreciated with ultrasonography (Babkine and Blond, 2009). It is a hyperechogenic line, composed by visceral and parietal pleura; the two can be differentiated only during a real-time examination, when breathing acts cause a sliding movement (Babkine and Blond, 2009). The lung parenchyma, instead, cannot be examined, because the air contained in the pulmonary lobes blocks the progression of the ultrasound waves (Babkine and Blond, 2009). The result is a reverberation artifact, composed by equidistant horizontal lines, also called A-lines (Babkine and Blond, 2009; Isciandro et al., 2014). The presence of bacterial or viral pathogenic agents in the low respiratory tracts is known to cause different tissue damages, including: consolidation, fibrosis, suppuration and abscess formation, pleural granularity and/or effusion (Panciera and Confer, 2010). Pleural diseases produce two main types of lesion: pleural effusion and pleural thickness or irregularity. At ultrasonography examination, the former is described as a separation of the two pleurae, with a liquid-like content between them, which can range from anechoic to more echoic, depending on its cellular content. The latter is difficult to evaluate objectively, but it can be defined by comparing healthy and ill sections of the lung (Babkine and Blond, 2009; Buczinski et al., 2014). Pneumonia is primarily characterized by consolidated lung, which is usually visible as hypoechoic structure with a texture that looks like liver parenchyma. Moreover, in this pathologically altered area, it is possible to observe fluid of alveolograms or bronchograms, and bronchoaerograms. The former two appear as anechoic structure, circular or tubular, respectively, with partially echogenic walls, which represent alveoli and bronchi filled with exudate of different type. Bronchoaerograms, instead, emerge as linear hyperechogenic structure in consolidated lung, and they represent bronchi filled with air. These structures often appear with a distal vertical reverb artifact (Flöck, 2004; Babkine and Blond, 2009). Other possible findings of the thoracic ultrasonography examination are comet tail artifacts (CT). They are vertical hyperechoic lines, emanated from pleura surface (Babkine and Blond, 2009; Ollivett et al., 2015). In human and small animals, the presence of CTs is considered as pathological sign only if more than three artifacts are found in one lung site. In that case, it reflects an increase of fluid in the interstitial space or in alveoli surrounded by air (Zhang et al., 2006; Isciandro et al., 2014; Ward et al., 2017). In bovine medicine, the CTs artifacts are found in presence of gas bubbles or when the pleura is thickened and/or irregular (Babkine and Blond, 2009; Ollivett et al., 2015). Moreover, a high amount of CTs was also described in case of pulmonary emphysema (Flöck, 2004). Consequently, the presence of few CTs artifact was not considered as pathological finding, even if sometimes they were the only lung alteration found in lungs with bacterial and viral infections (Ollivett et al., 2015; Ollivett and Buczinski, 2016). Thoracic ultrasonography showed higher accuracy (Se: 77%-94%; Sp: 93%-100%) than clinical score in dairy pre-weaned calves in identification of BRD affected animals, compared to both post-mortem examination and based on Bayesian latent class models (used to evaluate accuracy of diagnostic tests in the absence of a gold-standard comparison) (Rabeling et al., 1998; Ollivett et al.,

2015; Buczinski et al., 2016c). Moreover, thoracic ultrasonography allowed early identification of dairy heifers with an increase culling risk and a reduction of reproductive performance (Adams and Buczinski, 2016; Teixeira et al., 2017). Finally, the inter-operator agreement for detecting lung consolidation ranged from moderate to almost perfect, depending on the operator's experience (Buczinski et al., 2013). Most of the studies about thoracic ultrasonography were conducted on pre-weaned dairy calves. To the author knowledge, three studies regarding the utility of thoracic ultrasonography for diagnosis and prognosis outcome in beef cattle are reported (Abutarbush et al., 2012; Rademacher et al., 2014; Zeineldin et al., 2016). In two of these studies thoracic ultrasonography appeared to be a useful tool in the prediction of negative outcome or the diagnosis of BRD, while the third did not reported thoracic ultrasonography as a useful diagnostic/prognostic tools (Abutarbush et al., 2012; Rademacher et al., 2014; Zeineldin et al., 2016).

Acute phase proteins

Acute phase proteins (APP) are non-specified immune system components, produced by hepatocytes in response to Interleukin-1, Interleukin-6 and Tumor Necrosis Factor, whose release results from internal or external insult, such as infection, inflammation, surgical trauma or stress (Murata et al., 2004; Jones and Chowdhury, 2010). Acute phase proteins are defined positives, if their concentration increases following an insult, or negatives if their concentration decreases (Jones and Allison, 2007). In general, APP have high sensitivity, increasing quickly in case of infection, yet they have low specificity, considering that they increase accordingly to a number of stressful events, such as castration, transport or starvation (Eckersall and Bell, 2010; Abdallah et al., 2016). Consequently, the reliability of APP in the early detection of BRD is still under investigation. Haptoglobin (HP), Serum Amyloid A (SAA) and Fibrinogen (Fib) are positive proteins and they are the most commonly reported and evaluated in correlation with BRD (Abdallah et al., 2016). Serum Amyloid A increases and decreases rapidly (4 hours) after the initiation of inflammation or tissue damage (Petersen et al., 2004), while fibrinogen and haptoglobin reaches their peak 24-48 hours later, and haptoglobin can remain increased for 2 weeks (Petersen et al., 2004; Jones and Allison, 2007). This last seems to be correlated with diffuse lesions and bacterial infection and it appeared to be a good marker for the severity of damage and, in association with fibrinogen, it showed good sensitivity (80%) in the identification of animals that require anti-inflammatory treatment and high specificity in the identification of healthy animals (Humblet et al., 2004). Serum Amyloid A showed higher concentration compared to HP also after less important damages, such as viral infection (Petersen et al., 2004). In fact, the evaluation of SAA and HP together showed to be a useful parameter in order to discriminate between chronic and acute inflammation (Alsemgeest et al., 1994; Heegaard et al., 2000; Petersen et al., 2004). It has also been shown how APP could be an useful diagnostic tools for the identification of BRD affected animals, even when clinical signs were mild or moderate (Orro et al., 2011). Both SAA and HP were identified

as suitable health indicators in calves, but their increase has shown to coincide with the onset of clinical signs (Svensson et al., 2007). The use of these proteins as indicators of calves health could be a useful substitute to visual examination, since it would request an adequate sampling frequence and a constant evaluation during the production cycle, in order to have updated information about health status, but this solution would be expensive and of difficult application (Gånheim et al., 2007). Moreover, APP also showed to increase following stress as transport, weaning and commingling, making it impossible to use them as health indicators in the first fattening period, which is also that at higher risk of developing BRD (Qiu et al., 2007; Giannetto et al., 2011). Moreover, considering the presence of several commercial kits and the absence of a standard cut-off, their accuracy is difficult to be evaluated (Abdallah et al., 2016).

BRD ETIOLOGICAL DIAGNOSIS

Direct

Methods for detecting BRD pathogens are based on the isolation or culture of viruses and bacteria, respectively, or on the detection of viral and bacterial antigens or nucleic acids (Woolums, 2015b). These techniques are applied on nasal swabs, nasopharyngeal swabs, bronchoalveolar lavages, trans-tracheal aspiration fluids or on post-mortem tissue samples (Cooper and Brodersen, 2010).

Sampling techniques. Considering ante mortem sampling, whether a sampling method is best than the other has been debated. Nasal or nasopharyngeal swabs are easy and quick to perform, but they are believed to give less precise information, when compared to bronchoalveolar lavage or trans-tracheal aspirations. Nasal swabs are usually shorter and not-guarded, compared to nasopharyngeal swabs, even if the length depends on animals age and weight (Godinho et al., 2007; Wilson and Lakritz, 2015; Capik et al., 2017; Doyle et al., 2017). Bronchoalveolar lavage is frequently performed without sedation, by inserting a catheter trough the nares and into the trachea until it wedges in a bronchus (Capik et al., 2017; Doyle et al., 2017). The amount of saline solution introduced in the lungs is variable among studies, ranging from 50 ml to 240 ml, and consequently the re-aspired volume varied accordingly (Thomas et al., 2002a; Capik et al., 2017; Doyle et al., 2017). Trans-tracheal aspiration is more invasive and time-consuming, it could require animal sedation, and requires local anesthesia, clipping and sterile preparation of an area over the ventral trachea (Angen et al., 2009; Cooper and Brodersen, 2010; Doyle et al., 2017). Afterwards, a catheter is inserted through a 12/15 gauge cannula or trocar and pushed down the airways (Angen et al., 2009; Timsit et al., 2013; Doyle et al., 2017). Both the length of catheter and the amount of saline solution inoculated varied based on animal weight, age, and between studies. However, the catheters are usually shorter than those used in bronchoalveolar lavage; the amount of saline solution inoculated (20-60) and re-aspirated is lower (Angen et al., 2009; Cooper and Brodersen, 2010; Timsit et al., 2013; Doyle et al., 2017). Trans-tracheal aspiration has the advantage to bypass the upper respiratory ways, reducing possible contamination of upper respiratory ways by bacterial communities (Wilson and Lakritz, 2015). Nasal or nasopharyngeal swabs have been considered a valuable method for virus identification (Cooper and Brodersen, 2010), even if a recent study found a moderate agreement between nasal swabs and trans-tracheal aspiration samples for the identification of BRSV and BCoV (Doyle et al., 2017). More research have been performed concerning bacterial identification, with mixed results. Only one research group reported a very good agreement ($\kappa > 0.8$) between upper and lower respiratory tract samples for *M. haemolytica*, *P. multocida* and *M. bovis* (Doyle et al., 2017). Contrarily, others reported a moderate agreement ($\kappa = 0.40\text{-}0.60$) for *M. haemolytica*, *P. multocida*, *M. bovis*, *M. bovirhinis* and a slight agreement ($\kappa = 0.10\text{-}0.20$) for *H. somni*, even when the analysis was performed considering only BRD-affected animals or including healthy ones (Allen et al., 1991; Timsit et al., 2013; Van Driessche et al., 2017). Furthermore, a study focused on mycoplasma isolation found very low sensitivity of nasal swabs culture, compared to bronchoalveolar lavage culture,

for the identification of *M. bovis* and *M. bovirhinis* (Thomas et al., 2002b). Moreover, typing methods were applied in case of both upper and lower respiratory tract samples were positives for *M. haemolytica* or *P. multocida,* and the same type were identified in around the 70% of the matched pairs (DeRosa et al., 2000; Timsit et al., 2013). Godinho et al. (2007) found high positive predictive value, but low negative predictive value of nasopharyngeal swabs, compared to bronchoalveolar lavages, for the identification of *P. multocida* and *M. bovis*. They concluded, in accordance to Allen et al. (1991), that nasal or nasopharyngeal swabs could provide useful etiological information at group level, sampling a conveniently large group of animals (Godinho et al., 2007).

Bacterial and viral detection techniques. The sampling technique is not the only factor influencing the validity of results. In fact, different detection techniques proved to have different accuracy, execution time and cost. Viruses identification can be performed by mean of virus isolation, immunohistochemical (IHC) testing, indirect immunofluorescence (IFAT), antigen-capture enzyme immunoassay (EIA), enzyme-linked immunosorbent essay (ELISA) and PCR or RT (Reverse transcriptase)-PCR (Brodersen, 2010; Woolums, 2015b). BRSV and PI3 are very labile viruses, therefore isolation can easily result in false-negative (Brodersen, 2010; Ellis, 2010). Moreover, isolation is laborious and time-consuming (Brodersen, 2010). RT-PCR showed to be the most sensitive test for identification of BRSV, being able to detect the virus in a higher number of samples, compared to IFAT, EIA and ELISA, and it managed to be able to detect virus after a prolonged time following the onset of the clinical signs, compared to EIA (Vilcek et al., 1994; Larsen et al., 1999; Valarcher et al., 1999). Furthermore, in the recent years, a one-step multiplex RT-PCR was developed for the detection of BRSV, BHV-1 and PI3 (Thonur et al., 2012). It resulted to be rapid, cost-effective and more sensitive than IFAT and virus isolation for the identification of this 3 viruses (Thonur et al., 2012).

Bacterial detection is based on bacterial culture, IHC, IFAT, sandwich ELISA and PCR (Caswell et al., 2010; Woolums, 2015b). Mycoplasmas, in particular, require difficult and long culture techniques, thus the introduction of other detection techniques improved the identification of species belonging to this genus, notably *M. bovis* (Nicholas and Ayling, 2003; Woolums, 2015b). PCR demonstrate to have a higher rate of detection, when compared to bacterial culture for *P. multocida*, *M. haemolytica*, *H. somni* and *T. pyogenes*, and when compared to ELISA for *M. bovis* (Angen et al., 2009; Bell et al., 2014). However, Wisselink et al. (2017) found a good agreement between PCR and bacterial culture for *T. pyogenes*, a moderate agreement for *P. multocida* and *M. haemolytica*, and a slight agreement for *H. somni.* These findings are of particular interest for *H. somni*, which is rarely isolated with other pathogens, highlighting the difficulty in isolate this organism and the importance of PCR (Bell et al., 2014).

Indirect

Indirect diagnosis is based on the detection of antibodies in serum samples. Techniques used for antibodies detection included serum neutralization (SN) test, direct ELISA, direct immunofluorescence (IFA) and hemagglutination inhibition test (Woolums, 2015b). Antibodies detection alone does not give information about clinical status of the animal, because it can reflect a previous contact with the pathogen or a previous vaccination (Autio et al., 2007; Woolums, 2015b). Consequently, a second blood sampling has to be carried out 2 to 4 weeks after the first, in order to evaluate a possible seroconversion, which is represented by a fourfold increase in antibody titer (Woolums, 2015b). However, this allowed to have a retrospective diagnosis and can be, consequently, useful at the group level (Autio et al., 2007; Woolums, 2015b). Though it cannot be used to obtain a diagnosis in the first stage of the disease, serology can be more sensitive than culture in chronic infection of *M. bovis* or in subjects that received repeated treatment (Caswell et al., 2010). Furthermore, the detection of antibodies could be useful in epidemiological studies or to assess the evolution of the disease in outbreaks (Woolums, 2015b). For example, in a Texas study, the involvement of BVDV infection in *P. multocida* pneumonia was identified by means of seroconversion (Fulton et al., 2000). Moreover, in France, seroconversion for *M. bovis* highlighted the importance of this species in BRD development in veal calves (Arcangioli et al., 2008). A similar result was reported by Rosendal and Martin in Ontario (Rosendal and Martin, 1986). Finally, the presence of antibodies against viruses and bacteria at arrival at feedlot showed usefulness in the prediction of BRD development during the fattening period. In fact, Booker et al. (1999) reported that the presence of higher BVDV and *H. somni* antibodies titer at arrival decreased the incidence of BRD. Similar results were reported by Durham et al. (1991) and Hay et al. (2016), concerning the presence of BHV-1, BRSV, BVDV and PI3 antibodies.

TOWARDS NEXT GENERATION SEQUENCING AND THE RESPIRATORY MICROBIOTA CHARACTERIZATION

When only bacterial culture was available, bacterial phylogenetic studies were extremely limited, because the vast majority of species (> 99%) is not easy or impossible to be cultured and this technique does not allow to investigate the evolutionary relationships between different taxa (Pace, 1997). The introduction of biomolecular techniques, based on the study of DNA and RNA, improved the phylogenetic study of bacterial population, allowing to both discover not culturable bacteria species and to give information about their “evolutionary distance” (Pace, 1997; Handelsman and Handelsman, 2004). In particular, some fragments of ribosomal RNA (rRNA), i.e. the 16S rRNA gene, were selected and sequenced for phylogenetic analysis, due to their wide distribution, high amount of information and also high level of conservation (Lane et al., 1985; Hugenholtz et al., 1998; Handelsman and Handelsman, 2004). The phylogenetic study based on the analysis of 16S rRNA gene is called barcoding (Taberlet et al., 2012). The most diffuse sequencing method was the Sanger method (Sanger et al., 1977). With the further introduction of PCR and the identification of specific primers, the 16S rRNA genes could be entirely amplified and sequenced, thus accelerating the phylogenetic study of bacterial communities (Handelsman and Handelsman, 2004; Taberlet et al., 2012). An additional progress was reached following to the fragmentation and inclusion of the selected DNA in bacterial colonies, yielding to the whole genome sequencing, without the necessity of a previous PCR amplification (Fleischmann et al., 1995; Handelsman and Handelsman, 2004). Finally, in the last decade, new sequencing techniques were introduced, the so-called Next Generation Sequencing (NGS), able to produce a considerable amount of data (millions of reads) in a relatively short period of time (up to 7 days) (Metzker, 2010). Consequently, the Sanger method, now defined as a first-generation sequencing, had been outclassed by the second- and the third-generation sequencing, which could produce a dramatically higher number of reads within the same number of runs, in addition to a lower cost per read and over a shorter period of time (Ambardar et al., 2016). Likewise the Sanger method, the NGS sequencing can be applied to the whole genoma (shotgun sequencing) or on the 16S rRNA genes (metabarcoding) (Taberlet et al., 2012; Weinstock, 2012). Moreover, NGS allowed the study of bacterial communities as a whole, leading to identify possible variation in different niches or during time (Weinstock, 2012; de Steenhuijsen Piters et al., 2015; Timsit et al., 2016b). Even if these technologies are not flawless, they have led to remarkable findings, not only in the bacterial communities study, but also in the study of genomic and genomic alterations (Ambardar et al., 2016).

In 2007, the Human Microbiome Project started with the objective of characterizing the microbiota of different sites in the human body, such as upper respiratory tract, skin, gastrointestinal tract and urogenital tract, and then to study its possible correlation with disease status (Segal et al., 2014). The results showed that several microbes coexist in the human body, within specific body niches, and largely vary among people, although not necessarily associated with illness (Lloyd-Price et al., 2016). On the other hand, several non -infectious diseases, including inflammatory bowel disease, multiple sclerosis, diabetes, allergies, asthma, autism, and cancer were reconducted to alterations of the bacterial

communities (Lloyd-Price et al., 2016). Even if it was not included as one of the initial sites of the Human Microbiome Project, the lung was largely studied, leading to the discovery of bacterial communities in the healthy lung, previously considered sterile (Dickson et al., 2014a). Consequently, the model of pathogenesis of pneumonia has been revised as well: rather than considering it subsequent to the growth of a single bacterial species in a previously sterile area of the body, it is more likely correlated to alterations of the pre-existent bacterial community (Dickson et al., 2014a). In human medicine, many factors showed to influence the development of the respiratory microbiota, and therefore influence the health status of patients. Birth delivery method and early life nourishment (breast-feeding or formula feeding), for example, have been reported to shape the respiratory microbiota, with possible consequences on health, since cesarean section has been correlated with higher risk of developing asthma, whereas breastfeeding resulted in microbiota shifting towards species which seemed to have a protective influence against respiratory infections in the first months of life (Koppen et al., 2015). The respiratory microbiota showed to be influenced also by geographical locations, since British and American patients affected by cystic fibrosis differed in bacterial presence and relative abundance of certain species not traditionally associated with the disease (Stressmann et al., 2011). The presence of an upper and lower respiratory tract microbiota has been investigated also in horses, dogs and cattle (Bassis et al., 2015a; Holman et al., 2015b; Ericsson et al., 2016; Gaeta et al., 2017; Johnston et al., 2017; Zeineldin et al., 2017b). In the all the aforementioned species, the upper and lower respiratory tract significantly differed, suggesting the presence of a more homogeneous and less rich of bacterial communities lung microbiota (Bassis et al., 2015a; Ericsson et al., 2016; Zeineldin et al., 2017b). In calves, an environmental influence of the upper respiratory tract has been suggested, considering its variation following the relocation to a new environment (Timsit et al., 2016b). An example of the importance of this technology can be found in the fact that, NGS analysis of upper respiratory tract of both health and affected calves showed a significant higher abundance of *Lactobacillaceae* in health calves and following transportation (Holman et al., 2015a; Amat et al., 2016). This results drove to further researches that highlighted the ability of *Lactobacillaceae* to inhibit the growth and compet with *M. haemolytica* (Amat et al., 2017). These results, combined with the information acquired by human medicine, increase the interest in the prosecution of the studies on bovine respiratory microbiota, in order to reveal possible bacterial communities shaping factors and to develop new strategies for BRD control.

BRD ANTIMICROBIAL TREATMENT

Bovine Respiratory Disease treatment consists in the administration of the right antimicrobial, at the right dose and for a proper period of time (Woolums, 2015c). Several molecule classes are registered for BRD treatment both in North America and in Europe, including tetracyclines, macrolides, cephalosporines, fluoroquinolones, fenicoles and beta-lactams (EMA, 1996a; b, 1997; Lava et al., 2016; O'Connor et al., 2016). Different factors influence antimicrobial choice, including molecule efficacy, antimicrobial susceptibility test or minimal inhibitory concentration (MIC) test, clinical conditions of the animal, but also economic factors and the concern related to antimicrobial resistance (Jan et al., 2012; Woolums, 2015a). Antimicrobial efficacy may be evaluated in clinical trials by the veterinary practitioners, although this is not always applicable, besides, efficacy studies usually include one or two molecules and not all possible comparison are published (O'Connor et al., 2016). Consequently, O'Connor *et al*. performed a mixed treatment comparison meta-analysis, with the objective to help the veterinary practitioners in the choosing the most effective antimicrobials or, at least, to exclude the less efficacious ones (O'Connor et al., 2016). They reported tulathromycin as the most effective molecule, while ceftiofur, trimethoprim and oxytetracycline were the less effective (O'Connor et al., 2016).

Another factor to take into account when choosing the antimicrobial is represented by possible presence of resistance in BRD pathogens. This information may be obtained by performing a susceptibility test or MIC, yet a prompt treatment is often necessary, considering that early treatments improve the outcome (Lhermie et al., 2016). Therefore, data from literature can be extrapolated: tetracyclines are the antimicrobial family with the highest recorded number of bacterial resistance, followed, with a lesser extent, by macrolides, while the remaining classes accounted for a limited resistance prevalence (Portis et al., 2012; Lubbers and Hanzlicek, 2013). In order to reduce the development of resistance, the duration of treatment should provide contact between the antimicrobial and the pathogens for a suitable period, which should not be too brief nor prolonged (Apley, 2015). The extent of time is based on both pharmacokinetics characteristics and MIC for BRD pathogens (Apley, 2015). However, both in human and in veterinary medicine there is scant information over the correct antimicrobial therapy duration (Apley, 2015). Moreover, another important factor in defying a BRD treatment is the evaluation of treatment success, in order to decide whether to re-treat the animals or not. Recent studies conducted on single-injection antimicrobials (ceftiofur, tulathromycin, tilmicosin) showed that prolonged post-treatment interval may lead to a better outcome and reduce the number of animals that needed further antimicrobial therapy (Apley, 2015). This finding has been explained with the necessary recover following antimicrobial treatment, even if the latter had been successful, since the animal may require time to restore his physiological state (Apley, 2015). Finally, the administered antimicrobial dose is in extremely important: when dispensing a lower dose, compared that found on the product characteristic summary, the risk of antimicrobial resistance development and reduction of the treatment efficacy may increase (Zaheer et al., 2013; Catry et al., 2016).

BRD-associated antimicrobial use include not only the treatment of acute or chronic cases, but also the administration of these molecules for prophylactic or metaphylactic purposes (Jan et al., 2012; Ives and Richeson, 2015). Prophylactic use is described as the administration of antimicrobial to healthy animals at risk to develop BRD, albeit this risk is not always well defined and can include young age, stressful events, overcrowding or the introduction of new animals in the herds (Jan et al., 2012; EFSA/ECDC/EMA, 2017). Metaphylactic use is described as treatment in calves considered at "high-risk" of BRD development, such as the application of mass medication on healthy animals belonging to a herd/flock which were already interested by the disease (Ives and Richeson, 2015; Baptiste and Kyvsgaard, 2017; EFSA/ECDC/EMA, 2017). Edwards (2010), e.g. suggest that a metaphylactic treatment should be implemented when at least the 10% of calves have been treated for 2 or 3 consecutive days, or if the 25% of calves pulled were treated. Several studies reported a beneficial effect in the application of metaphylactic protocols in "high-risk" calves, also considering the difficulty in the early identification of sick animals (DeDonder and Apley, 2015). In Italy, molecules allowed for prophylactic or metaphylactic use include: injectable ampicillin, oral amoxicillin, injectable spiramycin, oral doxycycline, injectable florfenicol, injectable tulathromycin, oral tilmicosin, oral tylosin, injectable gamithromycin, injectable tildipirosin (Italian Ministry of Health). Considering the increasing concerning for antimicrobial resistance, the introduction of different methods for control of BRD are necessary. The efficacy of nitric oxide usage for the prevention and control of BRD has been evaluated (Regev-Shoshani et al., 2015; Timsit et al., 2017). Nitric oxide is an endogenously produced molecule with antibacterial and antiviral properties, which has a short half-life and, contrarily to antimicrobials, no residuals in meat production (Regev-Shoshani et al., 2015). Despite its efficacy in preventing BRD development, it was lower ability than tilmicosin in inhibiting the growth of *Pasteurellaceae* (Timsit et al., 2017). Other field studies for alternative methods of BRD control, including the research on the effect of probiotic bacteria, which proved be able to inhibit the growth of *M. haemolytica* in vitro (Amat et al., 2017).

ANTIMICROBIAL RESISTANCE AND ANTIMICROBIAL CONSUMPTION MONITORING

Despite the actual reduction of morbidity and mortality following metaphylactic treatment, when oral formulations are administered, a subsequent increase in bacterial resistance has been reported (Catharina et al., 2006; Catry et al., 2016). Moreover, species susceptible to form these resistances are not only bovine respiratory pathogens, but also enteric pathogens with a zoonotic potential, that may be transmitted to humans, such as *Campylobacter, Salmonella, E. coli, Enterococcus* spp. (Cameron and McAllister, 2016). The prevalence of antimicrobial resistance in these bacteria isolated from bovine fecal samples, carcasses at slaughterhouse and ground samples was very high (ranging from 40% to 95%) and involved antimicrobial classes or great importance for human medicine, such as fluoroquinolones and 3rd and 4th generations cephalosporines (Cameron and McAllister, 2016). Furthermore, Hao et al. reported data over the influence that antimicrobials generate in food-animal production as well as on the development of antimicrobial resistance in human pathogens (Hao et al., 2016). Furthermore, the European Food and Safety Authority (EFSA), the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) are working together with the objective of a large-scale surveillance of antimicrobial resistance and monitoring of antimicrobial usage. They reported that the use of certain antimicrobial in food-animal production, such as fluoroquinolones, tetracyclines and macrolides is correlated with the development of resistances in human *Campylobacter, Salmonella* and *E. coli* (ECDC/EFSA/EMA, 2017). These cause particular concern regarding fluoroquinolones and macrolides, which have been defined as *Highest Priority Critically Important Antimicrobials* by the World Health Organization (WHO), together with other bacterial classes like 3rd and 4th generation cephalosporines, meaning that they “are the sole, or ones of limited available therapies, to treat serious bacterial infections in people” and that “they are used to treat infections in people caused by either: bacteria that may be transmitted to humans from non- human sources, or bacteria that may acquire resistance genes from non- human sources” (World Health Organization, 2016). Beside the surveillance of antimicrobial resistance, the monitoring of antimicrobial usage is essential to become aware of the amount of molecules that is under use and what circumstances do not require it (ECDC/EFSA/EMA, 2017). EFSA, ECDC and EMA have published guidelines to follow in order to reduce antimicrobial usage, including the ban of prophylactic treatment, the reduction of metaphylactic treatment at situations in which they are needed, and the use of antimicrobials after susceptibility test (EFSA/ECDC/EMA, 2017). Many European countries, such as Denmark, Netherlands, Germany, Sweden, France, Belgium and United Kingdom have already implemented plans for the reduction of antimicrobial use and resistance, through banning or distributing the aforementioned guidelines (ECDC/EFSA/EMA, 2017). Denmark and Netherlands, the first countries to start the surveillance and monitoring program, have already registered a reduction in the antimicrobial resistance prevalence and in antimicrobial use (ECDC/EFSA/EMA, 2017). Italy had been reported among the three countries with the highest antimicrobial consumption in animal food-production, and very few solutions have been implemented in order to change the situation until July 2017, when a plan for antimicrobial resistance control, including both human and veterinary sectors, has been published

(Consiglio superiore della sanità, 2017). As already mentioned, in order to reduce antimicrobial consumption, the monitoring of antimicrobial use is fundamental. However, this could not be performed by only evaluating the amount of kg of antimicrobials used, considering the presence of metabolic differences among species, the wide range in animal body weights, and the fact that most of drugs are used in growing animals (Jensen et al., 2004). Two principal methods have been applied to express the antimicrobial usage in a standardized way, less influenced by different animal category characteristics: the number of Animal Daily Dose (ADD) and the milligrams of antimicrobials per population correction unit (PCU) (Jensen et al., 2004; Van Boeckel et al., 2015). The ADD is defined as the average maintenance dose for the main indication in a specified species and it is expressed in mg/kg (Jensen et al., 2004). The antimicrobial consumption is further expressed as the number of ADD used in a certain farms, regions or country in a determinate period. The number of ADD derives from the ratio between the total amount of milligrams of active substance and the total kilograms of animals at risk (MARAN, 2011). The estimation of weight is easier in standard animal production such as broiler or swine, but it is harder for cattle, where the weight widely varies among different production categories. Consequently, many weights have been proposed in literature or they have been directly calculated (Jensen et al., 2004; MARAN, 2017; Caucci et al., 2018). The PCU value is obtained multiplying the number of living animals by the number of production cycles and the ration between the carcass weight and the killing-out percentage (Van Boeckel et al., 2015). The majority of European data about antimicrobial consumption are reported as number of ADD used (Pardon et al., 2012b; Lava et al., 2016; ECDC/EFSA/EMA, 2017; MARAN, 2017).

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OBJECTIVES OF PHD PROJECT

In Italy, the meat production sector involves both veal and beef calves. Italian beef breeds (Chianina, Podolica, Marchigiana, Maremmana and Piedmontese) satisfiy only half of the total Italian meat demand. Consequently, every year many beef calves are imported from other European countries and fattened in fattening units, located mostly in the north of Italy. The present PhD projects were carried out in Piedmont region. Piedmontese bred is typical of the region and it is characterized by the presence of muscular hypertrophy, commonly known as “double muscling”. Piedmontese cattle are breed in cow-calf units and the fattening operations often take place in the same structures. Moreover, several fattening units are present on Piemontese territory, which serve mostly beef calves imported from France and belonging to Charolaise, Limousine and Blonde D’Acquitaine breed. In all these types of breeding, BRD is an important health issue. Considering the complexity of BRD pathogenesis and development, many factors have to be investigated in order to improve the knowledge of this disease. The identification of bacterial pathogens in the lower respiratory ways of clinically healthy calves raised questions about disease development. Moreover, certain pathogens not primarily correlated with BRD have already been isolated from lower and upper respiratory tract of BRD affected calves, but their role in the development of the disease is still under discussion. Therefore, the aim of the primary project of this PhD thesis was to deepen the knowledge of BRD etiopathogenesis, by mean of a new sequencing technique, the Next Generation Sequencing (NGS). In human medicine, the use of this technology lead to discover the non-sterility of the healthy lung, as well as relevant differencies in upper and lower respiratory tract populations, even if some similarities were found. Moreover, the results of these studies led to the assumption that the development of infectious diseases follows an alteration of bacterial population. At the time in which the PhD project was conceived, no similar studies on bovine respiratory microbiota had been found by the authors. The hypothesis of the primary project was that in bovine species as well, healthy lungs have a microbiota, which differs from the one of the upper respiratory tract. A further, subsequent hypothesis was that pneumonic lung microbiota significantly differed from the healthy lung one, not merely for the presence of pathogenic species, but in its whole composition.

However, as already stated, other factors have to be considered as associated to BRD. Transportation and weaning, for example, have been proposed as important predisposing factors for BRD development, thus partly explaining why beef calves transported in fattening units are among the categories at higher risk of BRD-related morbidity and mortality. Consequently, in collaboration with an association of veterinary practitioners working in beef calves fattening sectors, a secondary project was realized in fattening operations, where calves imported from France were examined and sampled at arrival. Data concerning transportation, provenience and BRD treatment in the first 60 days after arrival were then collected, in order to investigate possible risk factors for BRD development in the first days of the fattening period.

The importance of BRD in this type of production could be the cause of large antimicrobial consumption, as already reported for veal calf units. In the last years, both European and World Organizations focused

their attention on antimicrobial resistance correlated with anctimicrobial use in food animal production, highlighting the importance of antimicrobial use monitoring. Italy is reported as one of the highest antimicrobial user in food animal production in Europe. However, very little studies with the purpose of monitoring antimicrobial consumption were carried out in Italy, in comparison with other European Countries, such as Denmark and Netherlands, where antimicrobial consumption has been monitored for years and was accordingly reduced. Consequently, a secondary project was carried out in the same fattening operation units, with the aim of monitoring antimicrobial consumption and investigating possible risk factors correlated to BRD.

PRIMARY PROJECT: CHARACTERIZATION OF RESPIRATORY TRACT MICROBIOME IN HEALTHY AND BOVINE RESPIRATORY DISEASE (BRD) AFFECTED PIEDMONTESE CALVES

BACKGROUND

Bovine Respiratory Disease (BRD) is one of the main health issue in beef and dairy calves (Edwards, 2010; Stokka, 2010); it is a syndrome with a multifactorial etiology, and several different forms of clinical and microbiological manifestation have been identified (Panciera and Confer, 2010; Taylor et al., 2010). In European countries, its morbidity ranges from 2% to 22%, varying more when assessed at herd level (0%-90%), and it is the primary cause of mortality in calves (Svensson et al., 2006a; b; Stilwell et al., 2008; Assié et al., 2009; Gay and Barnouin, 2009; Brscic et al., 2012; Pardon et al., 2012a). Due to its high morbidity and mortality, BRD is addressed as a herd-scale problem. Due to the low accuracy of clinical examination, the application of antimicrobial prophylaxis or metaphylaxis, is considered to be among the most effective control practices (White and Renter, 2009; Nickell and White, 2010). However, metaphylaxis has become one of the main cause for the widespread use of antimicrobial in both beef and dairy calves, therefore raising public health concern over increased antibiotic resistance (Nickell and White, 2010; Portis et al., 2012; Catry et al., 2016). Thus, in order to increase the accuracy of the diagnosis, other diagnostic techniques have been introduced, one of them being thoracic ultrasonography, which was applied mainly on dairy calves, less on beef cattle (Abutarbush et al., 2012; Rademacher et al., 2014; Ollivett and Buczinski, 2016; Zeineldin et al., 2016). In the former, it showed higher accuracy than clinical examination and it appeared to be a convenient and non-invasive technique to be used in routinary practice (Buczinski et al., 2014; Ollivett et al., 2015).

Over the course of BRD, the most frequently identified bacterial pathogens by mean of culture-based methods are *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, and *Mycoplasma bovis* (Griffin, 2010). Referred as ubiquitous inhabitants of the bovine upper respiratory tract, these bacterial species can proliferate in the lungs, when inhaled during stressful events or viral infections (Griffin, 2010). However, they have also been isolated from the lower respiratory tract of calves not presenting clinical signs, hence raising further questions about their role in the etiopathogenesis of BRD (Allen et al., 1991; Angen et al., 2009). For this reason, a better understanding of the etiology of BRD is crucial, in order to improve animal health.

In the last decade, the use of high-throughput sequencing methods (Next Generation Sequencing, or NGS), coupled to DNA barcoding has advanced the comprehension of bacterial communities as a whole, compared to previous sequencing techniques (e.g. Sanger method), which allowed to sequence one fragment at time and were limited to the analysis of suitable isolated specimens (Taberlet et al., 2012). DNA metabarcoding allows the generation of multiple reads of the hypervariable regions of the 16S rRNA gene in a single run, thus yielding to a considerable amount of phylogenetic information in a

single experiment (Taberlet et al., 2012). The 16S rRNA gene metabarcoding approach, applied to lower respiratory tract samples from humans, led to the discovery that lungs are not sterile, even in healthy conditions (Segal et al., 2014). The finding of lung microbiota in healthy subjects was initially explained as a temporary contamination from the upper respiratory tract, either during sampling or by micro-aspiration (Charlson et al., 2011). However, recent studies have suggested that the lung microbiota could be considered an ecosystem and that its composition depends on the immigration, elimination, and reproduction rates of the microbial communities (Morris et al., 2013; Dickson et al., 2014a; b). Although the components of this ecosystem derive from the upper respiratory tract, they could be able to proliferate in the lungs, forming a self-sustaining lung microbiota (Morris et al., 2013; Dickson et al., 2014a; b). In addition, DNA metabarcoding analysis of pathological respiratory samples, led to the hypothesis that alteration of the lung microbiota could assume a key role in the pathogenesis of human lung diseases, including bacterial pneumonia (Dickson et al., 2014b; Huffnagle and Dickson, 2015).

In cattle, 16S rRNA gene metabarcoding to characterize the microbiota of the upper respiratory tract has been applied on nasal swab samples from dairy and beef calves (Holman et al., 2015b; a, 2017; Lima et al., 2016; Timsit et al., 2016, 2017a; Gaeta et al., 2017). Timsit et al. (2016) and Holman et al. (2017), reported that the upper respiratory tract microbiota of beef cattle is not stable in the first 40 days on feedlot, possibly explaining their higher susceptibility in developing BRD over this period. Moreover, Lima et al. (2016), found that the upper respiratory tract microbiota of dairy calves showed significant differences, depending on the animal clinical respiratory status. Mostly of the studies aimed at characterizing the respiratory microbiota in healthy or BRD-affected animals but defining the cases on the base of the clinical examination. Moreover, BRD-affected lung microbiota was characterized using post-mortem samples exclusively.

Consequently, the aim of the present study was to evaluate the accuracy of thoracic ultrasonography in beef calves for the diagnosis of BRD and compare it with bacterial culture outcome, as well as to characterize the microbiota of the upper and lower respiratory tracts, by applying 16S rRNA gene metabarcoding on nasal swab (NS) and trans-tracheal aspiration (TTA) samples from post-weaned Piedmontese calves, with or without ultrasonographic lung consolidation.

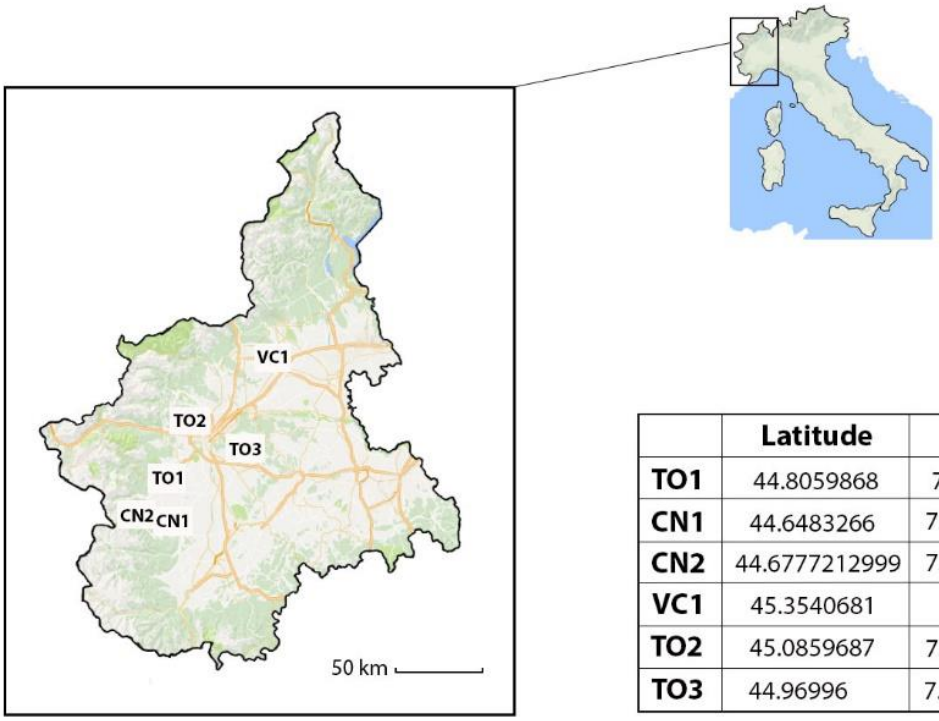
MATERIALS AND METHODS

The study protocol was established according to ethical recommendations and approved by the Animal Care Committee of the Department of Veterinary Sciences of the University of Turin. For each animal, the owner had to read and sign an informed owner consent, in order to authorize clinical procedures on his/her animals.

Sample population and sample collection

The study was carried out in Piedmont, northwestern Italy, from September 2015 to June 2016. Six farms were included, located in the provinces of Turin (TO), Cuneo (CN), and Vercelli (VC). The median distance as the crow flies between the farms was 50 km (min-max, 11–97 km) (map shown in figure 1). The farms were located in a limited area with similar geographical and climatic characteristics. The farms were all cow-calf operations of Piedmontese bred with the same animal management. Briefly, the calves were held with the mother until the end of the weaning period (5 months), then moved to multiple straw-bedded boxes (5–10 animals) with free access to water and feed (roughage and concentrate).

Figure 1: Geographical distribution of the 6 Piedmontese farms included in the study. TO = Turin CN = Cuneo VC = Vercelli.



	Latitude	Longitude
TO1	44.8059868	7.39083949999997
CN1	44.6483266	7.392150600000036
CN2	44.6777212999	7.279322200000024
VC1	45.3540681	8.0562228
TO2	45.0859687	7.539703000000031
TO3	44.96996	7.877819999999929

Both BRD-affected and healthy post-weaned Piedmontese calves were included in the study. They were selected from farms referred to the Veterinary Teaching Hospital of the University of Turin by

veterinary practitioners. Calves were examined at 3 experimental time points: T0 (day of inclusion), T1 (7 days after), T2 (21 days after). At each experimental time point, medical history, physical examination and thoracic ultrasonography data were collected via a standardized collection form for each calf. Moreover, nasal swabs (NS) and trans-tracheal aspiration (TTA) fluid samples were collected at T0 from each calf. Calves that had been treated in the week prior to examination or presented clinical signs suggestive of diseases different from BRD were excluded from the study.

A complete physical examination was performed for all animals, focusing on typical clinical signs of BRD: cough, nasal discharge, head tilt and abnormal sounds at thoracic auscultation (wheezes, crackles) (Master Classic II™, 3M Littmann® stethoscope, 3M, St. Paul, MN, USA).

Thoracic ultrasonography (TU) (MicroMaxx ®, SonoSite Inc., Bothell, WA) was performed with a convex probe at frequency of 2-5 MHz. Hair was not shaved, but 90% ethyl alcohol was applied, in order to reduce air presence and improve image quality. Systematic scanning was performed on both hemithoraxes from 10^{th} to 2^{nd} intercostal spaces, using the landmarks described by Ollivett and Buczinski (Ollivett and Buczinski, 2016). The probe was positioned in each intercostal space and moved dorso-ventrally. In order to standardize the recording of the lesions, both hemithoraxes were divided in four areas (craniodorsal, caudodorsal, cranioventral, caudoventral). The types of lesions noted were: B-lines or Comet Tail Artifacts (vertical artifacts arising from the pleura and extending to the edge of the screen) and lung consolidation (hypoechoic lung tissue with an echo texture similar to the liver parenchyma) (Zhang et al., 2006; Babkine and Blond, 2009).

Calves with lung consolidation at T0 were included as cases, while calves without lung consolidation at T0 were included as controls.

Nasal swab (NS) samples were collected using sterile swabs (17 cm, DrySwab, Copan S.p.A, Italy) from both nostrils of each calf after cleaning the nostrils with 90% ethyl alcohol. Trans-tracheal aspiration (TTA) was performed as previously described by Angen *et al* (Angen et al., 2009). Briefly, the animals were mildly sedated with intravenous injection of 0.05 mg/kg Xylasine (Rompun®, Bayer Healthcare, Germany), and an area of 3 × 3 cm about 7–10 cm caudal to the larynx was shaved and surgically prepared with 90% ethyl alcohol and iodophors. The area was desensitized with 4% procaine hydrochloride (Aticain®, A.T.I., Italy), and a longitudinal 1-cm incision was then placed in the midline directly above the trachea. A 12-G needle was used to perforate the trachea between two cartilage rings. A male dog urinary catheter (2 mm × 50 cm; Buster, sterile dog catheter, Kruuse, Germany) was introduced into the needle and pushed down into the airway for about 45 cm. Finally, a volume of 50 ml sterile 0.9% saline solution was injected through the catheter and immediately aspirated. To prevent inter-sample contamination, a new sterile kit and a new pair of sterile gloves were used for each calf.

For each calf, one nasal swab and one TTA aliquot were stored at -80°C for metabarcoding analysis. The remaining nasal swab and TTA fluid were submitted within 24 hours to the *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta* (IZSPLA) for bacterial and Mycoplasma cultures.

Bacterial and *Mycoplasma* cultures

For bacterial culture, specimens were inoculated onto Columbia Agar containing 5% sheep blood (Liofilchem, Italy), Chocolate Agar (Liofilchem, Italy) and onto MacConkey Agar plates and then incubated aerobically for 24 hours at 37°C. In addition, Columbia Agar containing 5 per cent sheep blood (Liofilchem, Italy) and Chocolate Agar (Liofilchem, Italy) plates were also inoculated and incubated in a CO_2-enriched (5%) atmosphere, for 72-96 hours at 37°C and monitored daily. Plates with a bacterial growth that had been considered significant (more representative and/or suggestive for respiratory pathogens), underwent to Genus and species-level identification using a biochemical test (API System or colorimetric Vitek 2GP card identification system, bioMérieux, France).

For *Mycoplasma* cultures, the specimens were streaked on PPLO Selective Agar (Microbiol, Italy), by rolling the swab over the agar surface and streaking for isolation. Plates were incubated in 5-10% CO_2 at 37 °C for up to 10 days. Furthermore, to enhance the recovery rate, PPLO Broth (Microbiol, Italy) was inoculated and incubated at 37 °C. for 4 days, then subcultured once onto PPLO Selective Agar Plate. Subcultures were examined daily for up to 4 days. When microscopic examination at 40-60X of inverted plates revealed the colony morphology of *Mycoplasma* (described as typical tiny "fried egg" colonies or “ground glass” colonies with a berry-like appearance penetrating the agar surface), suspected colonies were isolated, subcultured and submitted to identification by means of 16s rRNA sequencing (Benedetto et al., 2007).

DNA extraction and library preparation

DNA extraction. DNA was isolated using DNAzol® reagent (Invitrogen, Carlsbad, CA, USA), according to manufacturer’s instruction. Briefly, the nasal swabs (NS) were dipped in 500 µl of DNAzol® reagent immediately after collection, while the trans-tracheal aspiration (TTA) fluids were pelleted and added with 1 ml of DNAzol® reagent, after thawing at 4 °C. The samples were then repeatedly homogenized and incubated at 4°C for 18 hours. After DNA precipitation by means of ethanol, the pellets were rinsed twice. Finally, DNA was eluted in 30 µl of RNase and DNase free water. DNA concentration of each sample was determined using Qubit fluorimeter (Qubit®, Invitrogen) and normalized at 5 ng/µl. Samples with a lower concentration were not furtherly processed.

Library preparation. The normalized DNA was processed according to the 16S Metagenomic Sequencing Library Preparation protocol suggested by Illumina (Illumina, San Diego, CA, USA). Briefly, 12.5 ng of genomic DNA underwent an initial PCR with the 16S Amplicon PCR forward and reverse primers targeting the V3 and V4 regions of the 16S rRNA gene [29], followed by PCR cleanup with Agencourt Ampure XP (Beckman Coulter, Brea, CA, USA) magnetic beads, and an index PCR, followed by a second cleanup with magnetic beads.

Normalization was based on the average size of the library, with an Agilent High Sensitivity DNA Kit on a 2100 Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA) and a quantification with the NEBNext® Library Quant Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) normalization. The normalized libraries were eventually pooled and loaded for sequencing on an Illumina MiSeq platform with paired-end 2x300 bp protocol using a MiSeq® Reagent Kit ver. 3 (600 cycles) (Illumina).

Neither blank controls nor mock communities were included in the present study; however, in order to limit the influence of contamination by extraction and amplification on the analysis, all samples were processed using the same DNA extraction reagents, and the amplifications were conducted with the same reagent lots.

Statistical analysis

Calves that showed at least one clinical sign (spontaneous or induced cough, nasal discharge, abnormal ear position, abnormal lung sounds) of BRD were considered positive at the clinical examination; calves that had at least one area of lung consolidation were considered positive at lung ultrasonography. Finally, bacterial cultures where at least one potential pathogenic bacteria for BRD (*M. haemolytica, P. multocida, H. somni* and *Mycoplasma ssp.*) was identified were classified as positive. Statistical analysis was performed using software R, v. 3.4.0. The Cohen's κ coefficient was used in order to assess the agreement between the presence of lung consolidation on both sides of the thorax. The agreement was judged as slight when $0 \leq \kappa \leq 0.20$, fair when $0.21 \leq \kappa \leq 0.40$, moderate when $0.41 \leq \kappa \leq 0.60$, substantial when $0.61 \leq \kappa \leq 0.80$, and almost perfect when $0.81 \leq \kappa \leq 1$. The correlation between the presence of lung consolidation and comet tail artifacts was investigated by means of Fisher's exact test. Sensitivity (Se) and specificity (Sp), positive and negative predictive values (PPV, NPV) were assessed for clinical examination, in order to predict the presence of lung consolidation, while for thoracic ultrasonography the ability to predict the identification of at least one potential pathogenic bacteria in the TTA samples. Thoracic ultrasonography was evaluated considering both comet tail artifacts as pathological findings and without considering these findings as pathological. The significance was set at 0.05.

Bioinformatics analysis

Reads were processed for quality filtering (using Q30 as threshold), adapter and primer removal BBDuk2 ver. 36.14; mate pairing was performed with BBMerge ver. 9.00 (https://sourceforge.net/projects/bbmap/). The FASTA files were then processed using Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1 pipeline (Caporaso et al., 2010). In detail, paired reads

were merged in a single FASTA file with *multiple_split_libraries_fastq.py* script, which provides further quality filtering.

Operational Taxonomic Unit (OTU) picking was performed with the *pick_open_reference_otus.py* using the *UCLUST method and with 97% identity to the Greengenes (version gg_13_8) reference database, followed by de-novo clustering* (Edgar, 2010)*. Representative sequences were checked for de novo chimera detection* using ChimeraSlayer integrated in QIIME, and the chimeras were then filtered out from the OTU table (Haas et al., 2011). The alpha and beta diversities were computed with the *core_diversity_analyses.py* script, rarefying the samples at 2500 reads. Samples with less than 2500 reads were excluded from the statistical analysis because not representative. Phyla, genera and species abundance was reported as overall relative abundance, average relative abundance, and standard error of the mean (SEM).

Statistical support to the alpha diversity comparison between groups was assessed by nonparametric test with Monte Carlo permutations implemented in the *compare_alpha_diversity.py* script, while for the beta diversity comparison, QIIME wrapper *compare_categories.py* was applied with permutational multivariate analysis of variance (PERMANOVA, Adonis method from R package Vegan implemented in QIIME). Statistically significant differences in OTU frequencies based on non-normalized raw counts between the NS and TTA samples and between the TTA samples from animals with and without lung consolidation were assessed using the *differential_abundance.py* script that implements the R package DESeq2 (Love et al., 2014) , and *P*-values were adjusted (*P*adj) for multiple-testing with the false discovery rate (FDR) procedure of Benjamini and Hochberg (Benjamini and Hochberg, 1995). OTUs were considered differentially abundant if at $Padj \leq 0.05$ and if the estimated fold change was >1.5 or <1/1.5 (DiGiulio et al., 2015).

In order to evaluate the type I and type II errors and strengthen the results, a power analysis on the data grouped by sample type was performed, and by sample type and clinical signs according to the method presented by La Rosa and colleagues, implemented in the R package *HMP*, using the *MC.Xmcupo.statistics* function and 1000 Monte-Carlo experiments (La Rosa et al., 2012).

RESULTS

Physical examination and Ultrasonography

The number of calves selected from each farm ranged from 1 to 7, for a total of 22 calves (17 males and 5 females), aged from 5 to 14 months. Overall, only 2 out of 22 included calves underwent transportation before inclusion. At T0, 12/22 (55%) calves had at least one area of lung consolidation at thoracic ultrasonography (TU) and 9 (75%) of them showed concomitant clinical signs of BRD. Among subjects with lung consolidation, three had previously experienced BRD, although no specific treatment was recorded in the preceding week (calves 1, 3, 11), while the remaining nine were all diagnosed for BRD on the day of inclusion. TU consolidation areas were found on both hemi thoraces in 7/12 (58%) calves and involved the cranioventral areas in 86% of cases. The agreement between the left and the right side was moderate ($\kappa = 0.54$; 95% CI: 0.18 – 0.89; $p = 0.007$). Comet tail artifacts were present in six calves: four in association with lung consolidation and two without any other specific TU lesions. No more than one comet tail per area was found. The presence of comet tail artifacts did not seem correlated with the presence of lung consolidation ($P > 0.05$).

Further details of clinical examination and lung ultrasonography are reported in table 2.

Sensitivity of clinical examination to predict lung consolidation was 75% (95% CI: 43%-95%), while specificity was 100% (95% CI: 69%-100%). Positive and negative predictive value were 100% (95% CI: 66%-100%) and 77% (95% CI: 46%-95%), respectively (table 1).

Table 1: Sensitivity (Se), Specificity (Sp), Positive and Negative Predictive Value (PPV and NPV) of clinical examination to predict the presence of ultrasonography lung consolidation.

	Presence of lung consolidation	Absence of lung consolidation	Total	Se	Sp	PPV	NPV
≥ 1 clinical signs	9	0	9	75%	100%	100%	77%
No clinical signs	3	10	13				
Total	12	10	22				

Seven out of 10 calves without TU lung consolidation at T0 developed it within T1 (n=5) and T2 (n=2), and 5 (71%) of them showed also clinical signs. All the animals with TU lung consolidation at T0, continued to display it during the following experimental time points. Overall, 7 calves were treated with antibiotics during the study period and two calves died after the last follow-up: one was sent to the slaughterhouse due to constants relapses after 16 days, while the other died of natural death after 45 days.

Table 2. Calves data (origin, signalment, history, clinical examination and thoracic ultrasonography) on the day of inclusion.

ID = animal's identification number. Farm origin = farms are indicated with the provincial code (TO Turin, CN Cuneo, VC Vercelli) and progressively numerated. Sex: M = male, F = female. CT = Comet tail artifacts. TU = Thoracic ultrasonography.

ID	Farm	Age (months)	Previous BRD episodes	Cough	Nasal discharge	Head position	Abnormal lung sounds	CT	TU consolidation
1	TO1	14	3 months before sampling	Spontaneous and repeated	Cloudy and bilateral	Normal	Wheezes and crackles on the left side of the thorax	Yes	≥ 3 cms
2	CN1	6	No	Absent	Absent	Normal	Absent	No	No
3	CN1	6	1 months before sampling	Spontaneous and occasional	Absent	Normal	Wheezes and crackles on both side of the thorax	Yes	≥ 3 cms
4	CN1	6	No	Absent	Absent	Normal	Absent	Yes	≥ 3 cms
5	CN1	6	No	Absent	Absent	Normal	Absent	No	< 3 cms
6	CN2	6	No	Absent	Cloudy and unilateral	Normal	Absent	No	< 3 cms
7	CN2	6	No	Absent	Absent	Normal	Absent	No	No
8	CN2	6	No	Absent	Absent	Normal	Absent	No	No
9	CN2	6	No	Absent	Cloudy and bilateral	Normal	Wheezes and crackles on both side of the thorax	No	< 3 cms
10	VC1	5	No	Spontaneous and repeated	Absent	Normal	Wheezes and crackles on both side of the thorax	No	≥ 3 cms
11	VC1	5	2 months before sampling	Absent	Absent	Head tilt	Wheezes and crackles on both side of the thorax	No	< 3 cms
12	VC1	5	No	Absent	Absent	Normal	Absent	No	No
13	VC1	5	No	Absent	Absent	Normal	Wheezes and crackles on both side of the thorax	No	≥ 3 cms
14	TO2	6	No	Absent	Absent	Normal	Absent	Yes	No
15	TO2	6	No	Absent	Absent	Normal	Absent	No	No
16	TO2	6	No	Absent	Absent	Normal	Wheezes and crackles on the right side of the thorax	No	≥ 3 cms
17	TO2	6	No	Absent	Cloudy and bilateral	Normal	Absent	No	≥ 3 cms
18	TO2	6	No	Absent	Absent	Normal	Absent	Yes	< 3 cms
19	TO2	6	No	Absent	Absent	Normal	Absent	No	No
20	TO2	6	No	Absent	Absent	Normal	Absent	Yes	No
21	TO3	5	No	Absent	Absent	Normal	Absent	No	No
22	TO3	5	No	Absent	Absent	Normal	Absent	No	No

Bacterial culture

Four NS and four TTA samples were not submitted to the laboratory within 24 hours, therefore they were excluded from the analysis. Overall, potential pathogenic bacteria for BRD were found in 9/18 NS and in 14/18 TTA samples. The distribution of pathogens found in NS samples was the following: *Mycoplasma* spp. (45%) [*Mycoplasma bovis* (27%), *Mycoplasma bovirhinis* (9%), other *Mycoplasmas* spp. (9%)]. Moreover, bacterial BRD pathogens were found in 14/18 TTA samples: *Mycoplasma* ssp. (64%) [*Mycoplasma bovis* (36%), *Mycoplasma bovirhinis* (21%), other *Mycoplasmas* spp (7%)], *Pasteurella multocida* (43%), *Mannheimia haemolytica* (14%), *Trueperella pyogenes* (7%) (table 3).

Table 3. Bacterial culture results of NS and TTA samples. Bacteria considered pathogenic are shown underlined.

Animals' ID	TTA Bacterial results	NS bacterial results
1	*P. multocida; M. bovis*	*Acinetobacter lwofii;* mixed culture
2	*P. multocida*	*P. multocida; M.* spp
3	*P. multocida; M. bovis*	*Burkholderia cepacia, M. bovis, Enterobacter cloacae*
4	*Grimontia hollisae, M. bovis*	*Streptococcus sanguinis, M. bovis, E. cloacae*
5	*M. haemolytica*	*Alcaligenes faecalis, S. sanguinis*
6	*P. multocida*	*P. multocida*
7	Neg	*Pantoea agglomerans*
8	Neg	*Moraxella spp.*
9	*P. multocida*	Mixed culture
10	*M. bovirhinis*	Mixed culture
11	*M. bovis*	*Moraxella catharralis, Nocardia*
12	Neg	*Psychrobacter phenylpyruvicus, A. lwofii*
13	*M. bovirhinis, Nocardia*	*Nocardia*
14	*M. bovirhinis*	*M. catharralis, M. bovirhinis*
15	NA	NA
16	NA	NA
17	NA	NA
18	NA	NA
19	*M. bovis, M. haemolytica*	*M. bovis, M. catharralis, P. multocida*
20	*Mycoplasma spp., Trueparella pyogenes*	*M. catharralis, P. multocida*
21	*Moraxella ovis*	*Moraxella spp., M. haemolytica; Staphylococcus xylosus*
22	*P. multocida, M. ovis*	*P. multocida, Moraxella spp.*

Each calf with pathogenic bacteria in TTA samples had TUS lung consolidation at T0 (n=9) or developed it afterwards (n=5).

The classification of comet tail artifacts as pathological findings provided lung ultrasonography a higher accuracy (Se: 79%, Sp: 100%) in predicting the presence of pathogenic bacteria, compared to considering lung consolidation (Se: 64%, Sp: 100%) as sole pathological findings (table 4).

Table 4. Accuracy of thoracic ultrasonography (TU) and clinical examination in predicting the presence of potential pathogenic bacteria in the lower respiratory tract. Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) are reported as percentage (%) with their 95% confidence interval.

	Se (%)	**Sp (%)**	**PPV (%)**	**NPV (%)**
TU, considering CT artifacts as pathologic findings	79 (49-95)	100 (40-100)	100 (72-100)	57 (18-90)
TU, considering pathologic findings only consolidation	64 (35-87)	100 (40-100)	100 (66-100)	44 (14-79)
Clinical examination	50 (23-77)	100 (40-100)	100 (59-100)	36 (11-69)

Genetic analysis

Nasal swab samples for metagenomic analysis were collected from 17 out of 22 animals, and TTA samples were collected from all animals. DNA was successfully extracted at a concentration higher than 5 ng/µl from 32 samples (13 NS and 19 TTA fluid samples). In one of the 12 animals presenting TU lung consolidation (calf 9), the DNA concentration was below 5 ng/µl in both the NS and TTA samples; this animal was excluded from sequencing. At least one sample (NS or TTA) from 21 out of 22 calves was available for analysis. Subsequently to primer and quality trimming and pair merging, the total read count was 3,482,819, with an average read length of 407 ± 80 bp. After application of the *multiple_split_libraries_fastq.py* script to merge all the sequences in a single file, with further quality checks, the read number was 2,813,497. The total reads classified in the OTU table was 1,645,584, divided in 526,932 from the NS samples (median 24,072, min-max 159–167,198) and 1,118,652 from the TTA fluid samples (median 53,494, min-max 189–143,886). The number of assigned reads was below 2500 in 4 (2 NS and 2 TTA) out of 32 samples, and they were excluded from the analysis. Therefore, the final analysis was performed on 28 samples (11 NS and 17 TTA) from 19 calves. Of these 28 samples, 18 (64.3%) were matched samples from the same animal (9 NS and 9 TTA) (table 5). Finally, the reads obtained from these 28 samples were classified in 4368 OTUs (median 226.5, min-max 44–2502). The median (min-max) of the OTUs was 957 (495–2502) in the NS samples and 139 (44–719) in the TTA samples. A total of 810 unique sequences were classified as chimeric sequences and therefore removed from the OTU table before analysis.

Table 5. Data on farm origin, presence of lung consolidation and number of reads found in each sample. Farm origin = farms are indicated with the provincial code (TO = Turin, CN = Cuneo, VC = Vercelli) and progressively numerated;

TTA = trans-tracheal aspiration sample.

NS = nasal swab sample.

NP = not performed.

DNA < 5 ng/µl = samples with DNA concentration lower than 5 ng/µl and not sequenced.

** = samples with less than 2500 reads excluded from the final analysis.*

Animal's ID	**Farm**	**Lung consolidation T0**	**N° reads per NS sample**	**N° reads per TTA sample**
1	TO1	≥ 3 cms	NP	53,494
2	CN1	No	NP	7,902
3	CN1	≥ 3 cms	NP	47,115
4	CN1	≥ 3 cms	NP	18,855
5	CN1	< 3 cms	NP	81,564
6	CN2	< 3 cms	36,160	129,750
7	CN2	No	28,543	118,454
8	CN2	No	31,232	61,778
9	CN2	< 3 cms	DNA < 5 ng/µl	DNA < 5ng/µl
10	VC1	≥ 3 cms	40,694	142,459
11	VC1	< 3 cms	12,952	DNA < 5ng/µl
12	VC1	No	5,988	DNA < 5ng/µl
13	VC1	≥ 3 cms	24,070	26,910
14	TO2	No	DNA < 5 ng/µl	68,954
15	TO2	No	20,464	40,790
16	TO2	≥ 3 cms	167,198	107,679
17	TO2	≥ 3 cms	DNA < 5 ng/µl	143,886
18	TO2	< 3 cms	3,932	55,630
19	TO2	No	DNA < 5 ng/µl	1,825*
20	TO2	No	154,004	4,585
21	TO3	No	1,536*	6,833
22	TO3	No	159*	189*

Ten (58%) out of the 17 calves, whose TTA fluid samples were included in the final analysis, had TUS lung consolidation at T0. Median OTUs were 139 (46 – 255) in TTA samples from calves without lung consolidation at T0 and 136 (44 – 719) in TTA samples from calves with lung consolidation at T0.

Phylum composition

Overall composition. Overall, 29 phyla were identified. The microbial community of the samples was structured as follows: Tenericutes (61.8%, 59% ± 5.6%), Proteobacteria (19%, 15.6% ± 3.2%), Firmicutes (6.5%, 8.1% ± 2.2%), Bacteroidetes (5.6%, 7.2% ± 1.8%), Actinobacteria (3.8%, 7.1% ± 2%), Fusobacteria (2.8%, 1.6% ± 1.1%), others (0.3%, 0.4% ± 0.1%), and unassigned (0.2%, 1.1% ± 0.9%). Of the 29 phyla identified in the NS samples, the most abundant were Proteobacteria (36.1%, 21.9% ± 5.3%), Tenericutes (27.7%, 35.4% ± 6.9%), Firmicutes (18.4%, 19.3% ± 3.3%), Bacteroidetes (10.1%, 10.8% ± 2.5%), and Actinobacteria (6.3%, 8.9% ± 1.6%). Only 21 of 29 phyla were identified in the TTA fluid samples, and the most abundant were Tenericutes (77.9%, 74.3% ± 5.6%), Proteobacteria (11.0%, 11.4% ± 3.7%), Fusobacteria (4.2%, 2.5% ± 1.7%), Bacteroidetes (3.5%, 4.8% ± 2.4%), and Actinobacteria (2.6%, 5.9% ± 3.2%). Figure 2 presents the phylum abundance and composition in the upper and lower respiratory tracts.

TTA Composition based on presence/absence of lung consolidation. Tenericutes was the most abundant phylum both in calves with (74.1%, 71.8% ± 7.3%) or without (88%, 77.9% ± 2.7%) lung consolidation. In calves with lung consolidation other phyla were Proteobacteria (14.6%, 17.1% ± 5.5%), Fusobacteria (5.7%, 3.7% ± 2.9%), Bacteroidetes (3.9%, 5.2% ± 3.9%) and Firmicutes (1.1%, 1.2% ± 0.7%); while in calves without lung consolidation other phyla were Actinobacteria (7.8%, 12.8% ± 2.1%), Bacteroidetes (2.5%, 4.3% ± 0.7%) and Proteobacteria (1.3%, 3.4% ± 0.6%). The distribution of phyla in TTA of calves with or without lung consolidation are shown in figure 3.

Figure 2. Average relative abundance of phyla in the nasal swab (NS) and trans-tracheal aspiration (TTA) samples. Only phyla with a relative abundance higher than 1% in at least one sample type were represented. Red columns represent TTA (n = 17), while blue columns represent NS (n = 11) samples. The bars represent the standard error of the mean.

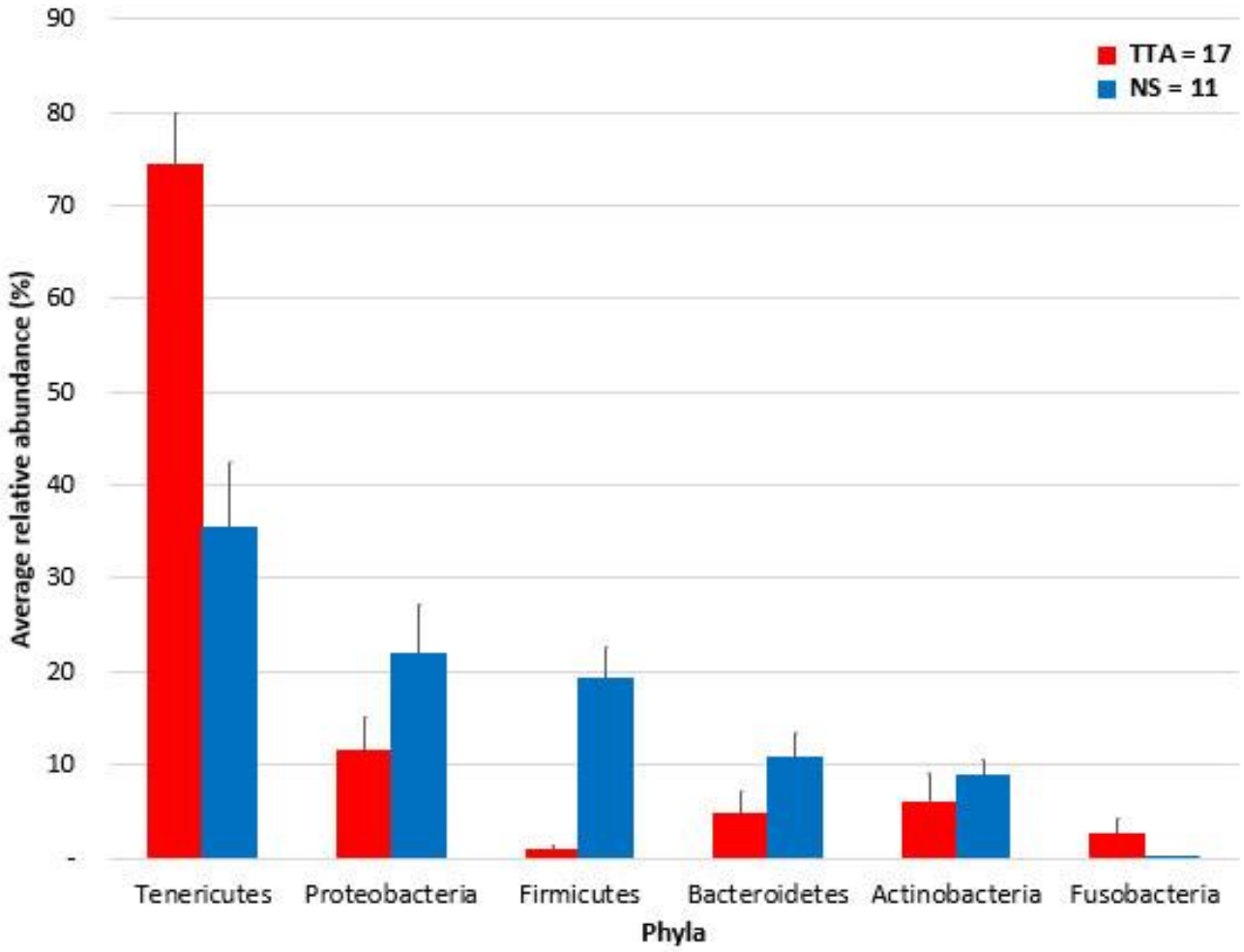
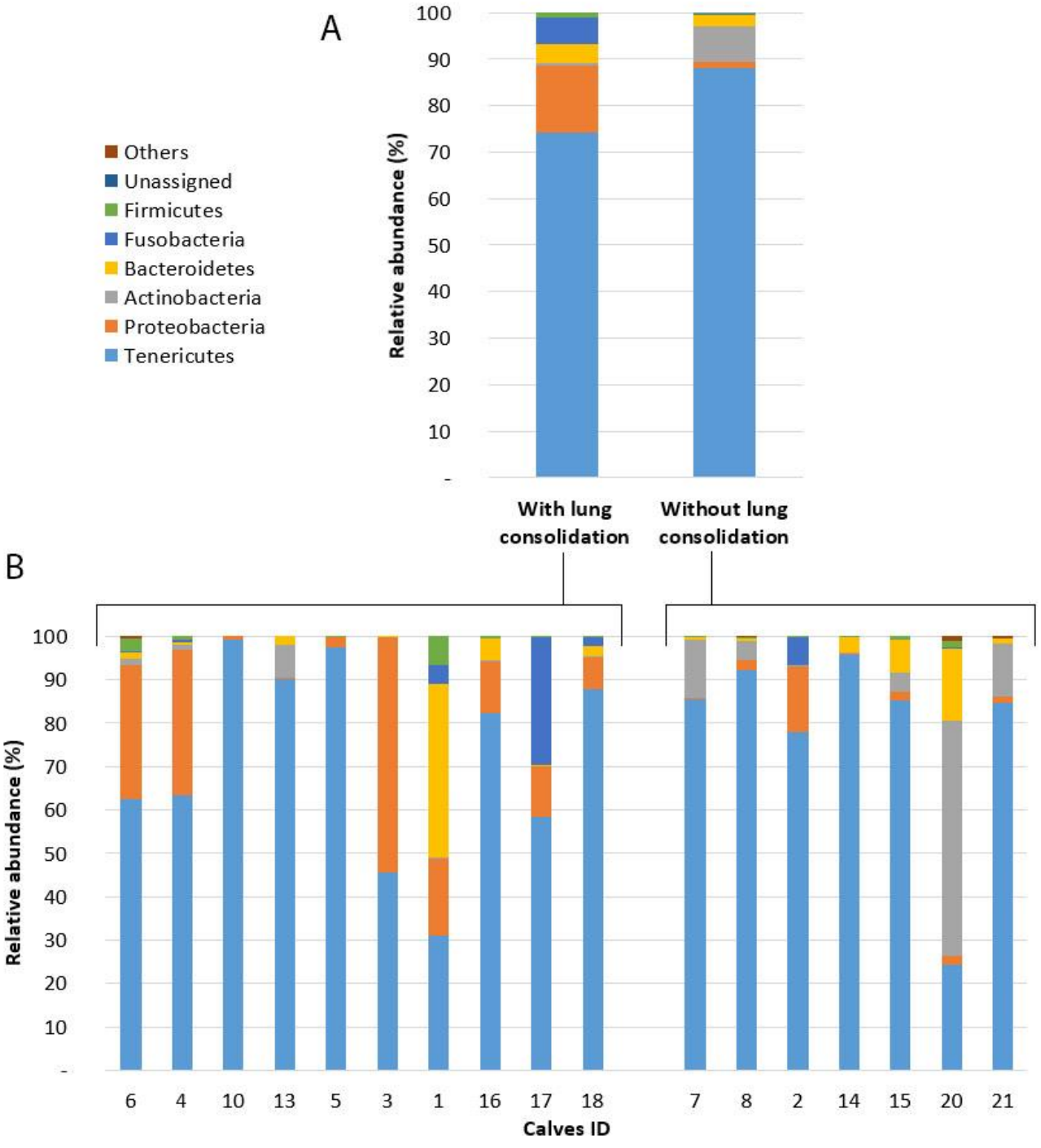


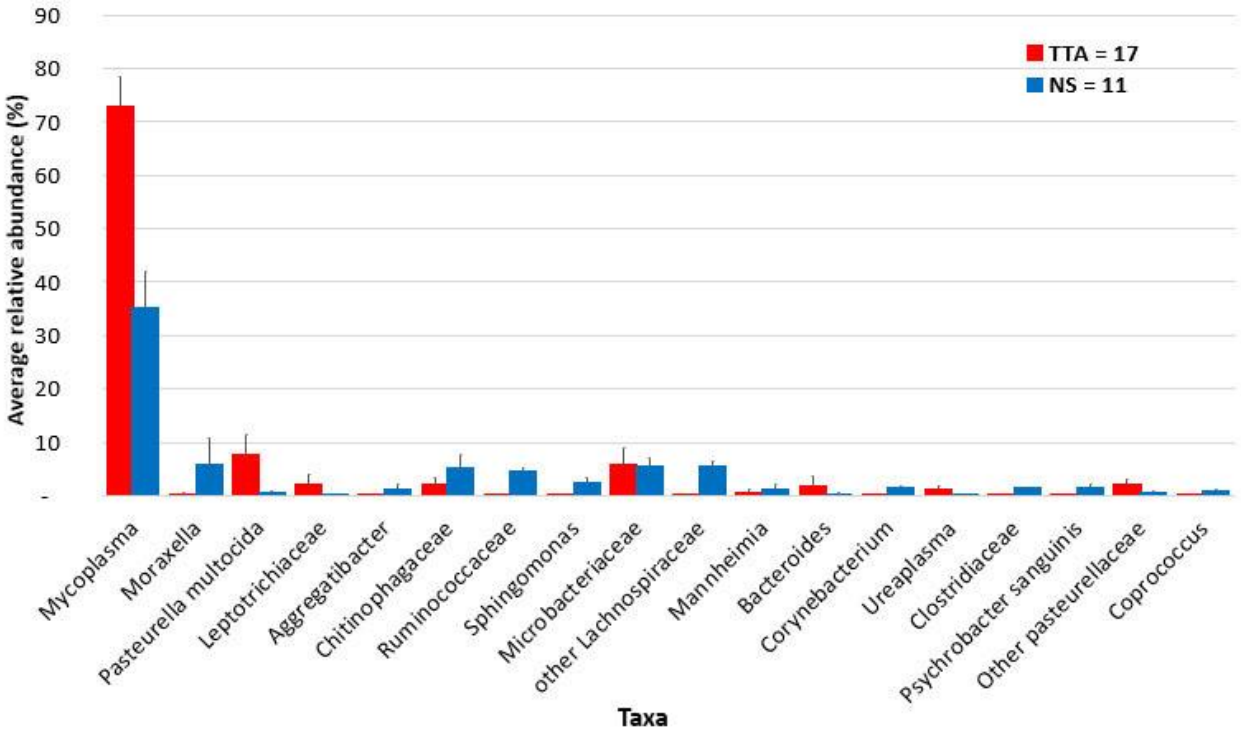
Figure 3: Relative abundance of most abundant phyla (relative abundance > 1%) identified in trans-tracheal aspiration (TTA) samples was reported both individually (B) and grouped (A) for calves with (n = 10) and without (n = 7) lung consolidation.



Taxa composition

Overall composition. A total of 305 genera were identified: 289 in the NS samples and 182 in the TTA samples. Moreover, 100 species were identified: 55 in TTA and 93 in NS. The most abundant taxa were: *Mycoplasma* (60.7%, 58.1% ± 8.9%), *Moraxella* (5.5%, 2.6% ± 3%), *Pasteurella multocida* (5.2%, 4.9% ± 3.7%). In addition, other taxa with a relative abundance higher than 1% were identified: *Leptotrichiaceae* (2.7%, 1.4% ± 1.1%), *Microbacteriaceae* (2.5%, 5.6% ± 2%), *Chitinophagaceae* (2%, 3.4% ± 1.1%), *Sphingomonas* (1.2%, 1.1% ± 0.6%), *Ruminococcaceae* (1.2%, 1.4% ± 0.4%), *Mannheimia* (1.2%, 0.9% ± 0.9%), *Aggregatibacter* (1.2%, 0.5% ± 0.6%), and *Bacteroides* (1.1%, 1.2% ± 1.7%). *Mycoplasma* was the most abundant genus in both the NS and the TTA fluid samples, with a relative abundance of 27.2% (35.1% ± 6.9%) and 76.5% (72.9% ± 5.5%) respectively, followed by *Moraxella* in the NS samples (16.6%, 5.9% ± 4.8%) and by *Pasteurella multocida* in the TTA samples (7.3%, 7.6% ± 3.7%). A few other genera with an abundance > 1% were also found in the NS samples [*Aggregatibacter* 3.6% (1.2% ± 1%), *Sphingomonas* 3.4% (2.5% ± 0.8%), *Corynebacterium* 1.3% (1.6% ± 0.2%), *Psychrobacter* 1.2% (1.6% ± 0.6%), *Coprococcus* 1% (1% ± 0.2%)] and the TTA samples [*Mannheimia* 1.6% (0.7% ± 0.6%), *Bacteroides* 1.5% (1.8% ± 1.8%), *Ureaplasma* 1.3% (1.2% ± 0.6%)] (figure 4).

Figure 4. Average relative abundance of taxa in the nasal swab (NS) and trans-tracheal aspiration (TTA) samples. Only taxa with a relative abundance higher than 1% in at least one sample type were represented. Red columns represent TTA (n = 17), while blue columns represent NS (n = 11) samples. The bars represent the standard error of the mean.



Besides *Mycoplasma*, present in all samples, *Delftia*, *Sphingomonas*, and *Agrobacterium* composed the core biota of 90% of the samples. Twelve taxa were present in all NS samples, including seven orders (*Aeromonadales*, *Actinomycetales*, *Clostridiales*, *Flavobacteriales*, *Mycoplasmatales*, *Saprospirales*, and *Sphingomonadales*) and four genera (*Succinivibrio*, *Mycoplasma*, *Sphingomonas*, and *Corynebacterium*).

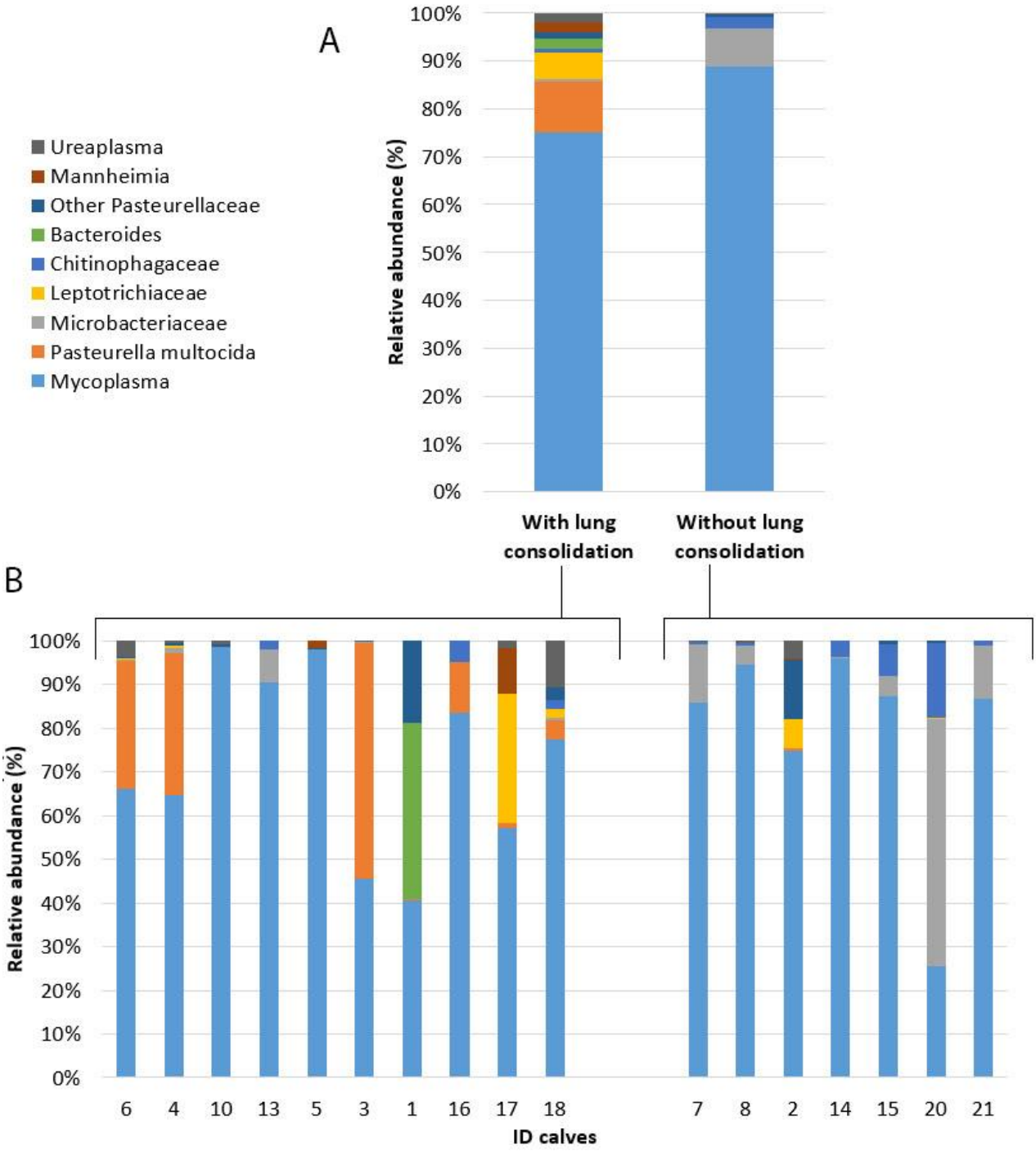
Overall, 7 species and 16 genera were found only in the TTA samples and 45 species and 123 genera only in the NS samples. The complete list of all genera and species identified in TTA and NS is reported in Appendix 1: Table S1 and table S2.

TTA Composition based on presence/absence of lung consolidation. The phylum Tenericutes was composed mainly by the genus *Mycoplasma* in TTA of both calves with (97.6%, 97.7% ± 1.3%) or without (99.7%, 99.1% ± 0.7%) lung consolidation. Moreover, in TTA of calves with lung consolidation genus *Ureaplasma* composed the phylum Tenericutes with a relative abundance higher than 1% (2.3%, 2.2% ± 1.2%). In TTA of calves with lung consolidation, the second most important phylum, Proteobacteria, was composed mainly by *Pasteurella multocida* (69.5%, 44.7% ± 14.5%), *Mannheimia* (14.1%, 17.6% ± 11.7%), other *Pasteurellaceae* (8.8%, 21.6% ± 11.6%), *Sphingomonas* (1.6%, 3.5% ± 2.9%) and *Moraxella* (1.2%, 1.4% ± 1.2%). In TTA of calves without lung consolidation, the second most important phylum, Actinobacteria, was composed for the 99.1% (91.2% ± 5.2%) by *Microbacteriaceae.*

Even if the taxa with the higher relative abundance are variable across the TTA samples, the core microbiota of the TTA fluids collected from calves with lung consolidation was composed by: *Mycoplasma* (72.3%; 70.1% ± 7.2%), *Pasteurella multocida* (10.1%; 12.8% ± 5.9%), *Leptotrichiaceae* (5.4%; 3.2% ± 2.9%), *Mannheimia* (2.1%; 1.2% ± 1%), *Bacteroides* (2%; 3.1% ± 3.1%), *Ureaplasma* (1.7%; 1.7% ± 1.1%), other *Pasteurellaceae* (1.3%; 1.8% ± 1.4%). However, in the TTA fluids of calves without lung consolidation, only three taxa were present with an abundance higher than 1%: *Mycoplasma* (87.7%; 77.2% ± 9.2%), *Microbacteriaceae* (7.7%; 12.6% ± 7.1%) and *Chitinophagaceae* (2.4%; 4.2% ± 2.2%). The distribution of most abundant taxa in TTA of calves with or without lung consolidation are shown in figure 5.

Overall, 17 species, 69 genera, 28 families were found in calves with consolidation and 9 species, while 17 genera and 8 families in calves without consolidation.

Figure 5. Relative abundance of most abundant taxa (relative abundance > 1%) identified in trans-tracheal aspiration (TTA) samples was reported individually (B) and grouped (A) for calves with (n = 10) and without (n = 7) lung consolidation.



Comparison of bacterial composition between TTA fluid and NS samples

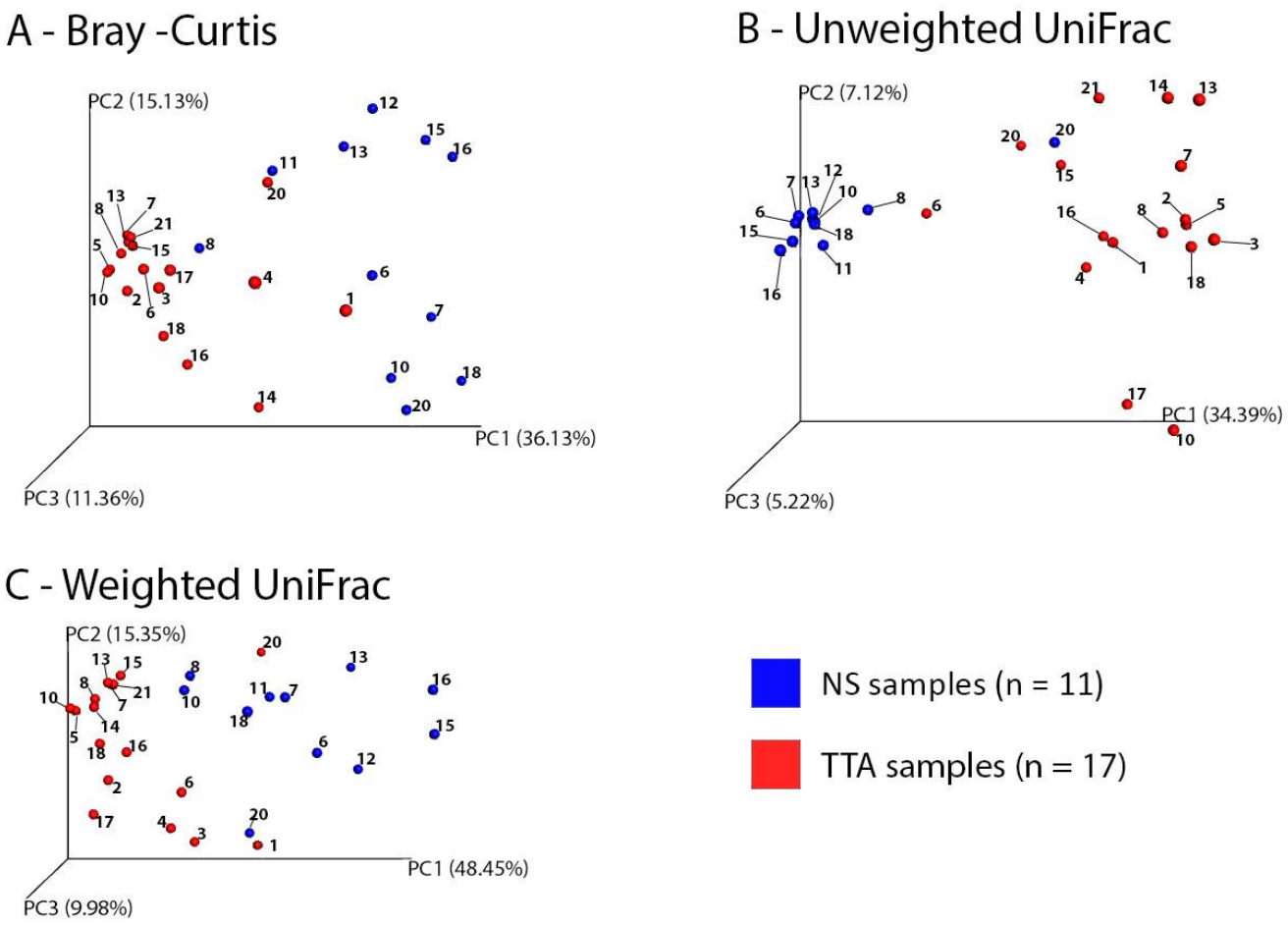
Alpha diversity values are reported in Table 6. Good's coverage estimates with a rarefaction at 2500 was 91.6% ± 2.9% for the NS and 99% ± 0.9% for the TTA fluid samples. The alpha diversity indices and rarefaction curves of each sample are reported in Appendix 2.

Table 6. Alpha diversity indexes calculated for the nasal swab (NS) and trans-tracheal aspiration (TTA) samples. Chao1 index, observed species, Shannon's diversity index, and Simpson index values are reported as mean ± standard error.

	TTA = 17	**NS = 11**	***P* value**
Chao1 index	95.62 ± 78.48	720.74 ± 225.22	0.001
Observed species	40.57 ± 35.21	395.30 ± 152.62	0.001
Shannon index	1.46 ± 0.83	5.14 ± 1.74	0.001
Simpson index	0.45 ± 0.24	0.82 ± 0.13	0.001

There was a statistically significant difference in Shannon's diversity index, Simpson index, Chao1 index and in Observed species between the TTA fluids and the NS samples ($P < 0.01$). The microbial composition of the upper and lower respiratory tracts was compared by Bray-Curtis dissimilarity, weighted UniFrac, and unweighted UniFrac phylogenetic distances. The difference between the two bacterial communities was statistically significant as assessed by Adonis ($P < 0.01$), based on the three different distance matrices. Principal coordinates analysis (PCoA) plots of the methods are shown in figure 6. The type I error with a significance at 0.05 was < 0.001, as was the type II error, providing a power > 90%.

Figure 6. Principal coordinates analysis (PCoA) 3D images. PCoA was performed using Bray-Curtis dissimilarity (A), unweighted UniFrac (B), and weighted UniFrac (C) distance matrices. Each sample is represented by a point with nasal swabs (NS = 11) in blue and trans-tracheal aspiration (TTA = 17) in red. The points are labelled with the identification numbers of the animals as reported in table 1. The clustering observed between the NS and TTA samples indicates differences in the microbial compositions of these sampling sites.



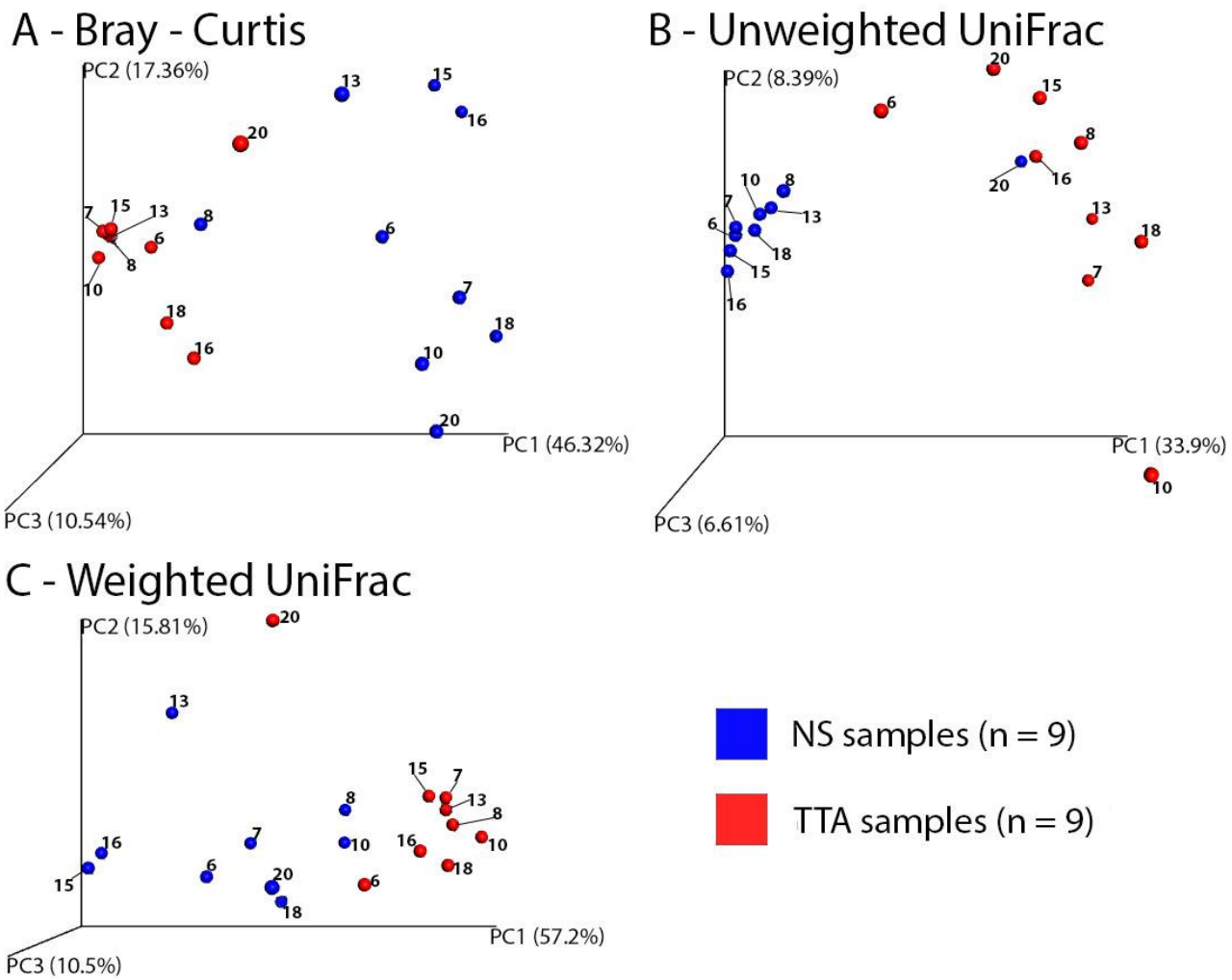
Overall, there were 240 taxa (1 class, 15 orders, 58 families, 128 genera and 38 species) with a statistically significant difference in abundance, (as assessed by DESeq2 analysis), with 17 more abundant in the TTA microbiota and 223 in the NS one (Appendix 3: TableS4).

In order to evaluate the presence of possible bias, a sub-analysis was performed with the only 9 calves with matching samples. The differences in alpha and beta diversity between NS and TTA samples were maintained (table 7; figure 7).

Table 7. Alpha diversity indexes calculated for the nasal swab (NS) and trans-tracheal aspiration (TTA) samples of the 9 calves with matching samples. Chao1 index, Observed species, Shannon's diversity index, and Simpson index values are reported as mean ± standard error.

	TTA = 9	**NS = 9**	***P* value**
Chao1 index	113.2 ± 96.6	715.9 ± 260.3	0.001
Observed species	47.8 ± 46.1	394.1 ± 169.7	0.001
Shannon index	1.2 ± 0.7	5.1 ± 1.9	0.001
Simpson index	0.37 ± 0.21	0.81 ± 0.14	0.001

Figure 7. Principal coordinates analysis (PCoA) 3D images of the 9 calves with matching samples. PCoA was performed using Bray-Curtis dissimilarity (A), unweighted UniFrac (B), and weighted UniFrac (C) distance matrices. Each sample is represented by a point with nasal swabs (NS = 9) in blue and trans-tracheal aspiration (TTA = 9) in red. The points are labelled with the identification numbers of the animals as reported in table 1. The clustering observed between the NS and TTA samples indicates differences in the microbial compositions of these sampling sites.



Correlation of microbiota composition in relation to farm of origin and thoracic ultrasonography findings.

Comparison of the microbiota composition of the TTA samples by farm of origin showed no statistically significant difference (Adonis on either Bray-Curtis dissimilarity, weighted UniFrac, or unweighted UniFrac distance; $P > 0.05$). It was not possible to estimate the type I and II errors because one group had size = 1. Similarly, no statistical difference in the microbiota composition of the NS samples was found when two statistical methods (Adonis on Bray-Curtis dissimilarity and weighted UniFrac distances; $P > 0.05$) were applied, except when compared based on unweighted UniFrac distance ($P = 0.05$). The type I error with a significance at 0.05 was < 0.001, as well as the type II error, providing a power > 90%. Finally, no difference in both alpha and beta diversity was found between the presence or absence of TU consolidation and microbial composition of the TTA samples (Adonis on Bray-Curtis dissimilarity, weighted UniFrac, or unweighted UniFrac distance; $P > 0.05$, power > 90%).

No differences were found among samples in alpha diversity based on thoracic ultrasonography findings ($P > 0.05$). Additionally, the presence of lung consolidation did not lead to a statistical difference in microbial communities, neither based on Bray Curtis, unweighted or weighted Unifrac distance ($P > 0.05$).

Nevertheless, some statistical differences were found comparing the taxonomic categories with the DeSeq test. Some of the taxonomic categories composing the core microbiota of TTA fluids of calves with lung consolidation (*Pasteurella multocida, Mannheimia, Leptotrichiaceae* and *Ureaplasma*) were significantly more abundant in this group of calves compared with the ones without lung consolidation ($P < 0.05$). Furthermore, other three genera and one family were significantly more abundant in calves with lung consolidation ($P < 0.05$): *Fusobacterium, Actinobacillus, Streptococcus and Mycosplasmataceae*. No categories were significantly more represented in TTA of calves without lung consolidation ($P > 0.05$). Nevertheless, there was a tendency for *Microbacteriaceae*, the most abundant OTUs in TTA of calves without lung lesions beyond *Mycoplasma*, to be more abundant in the TTA fluids of calves without lung consolidation ($P < 0.1$). (details in Appendix 4: table S5).

DISCUSSION

Clinical examination and ultrasonography

Thoracic ultrasonography was applied mostly on dairy or veal calves of 3 months of age or less. In these animals category, this technique showed high accuracy in the identification of lung lesions found at post-mortem examination (Rabeling et al., 1998; Ollivett et al., 2015). Moreover, it has been proposed as a screening method for early BRD identification in pre-weaned dairy calves, due to its higher accuracy, compared to the clinical examination (Berman et al., 2014; Buczinski et al., 2015; Ollivett and Buczinski, 2016). In beef cattle, there are few studies about the application of ultrasonography as a screening method, but they also reported the probability of found ultrasonographic lesions in clinically healthy animals (Abutarbush et al., 2012; Rademacher et al., 2014; Zeineldin et al., 2016). Nevertheless, opposing to the results of the present study, subjects with clinical signs and no ultrasonographic lung consolidation were reported in beef calves. However, in these studies ultrasonographic examination did not include the cranial lobes of the lung (Rademacher et al., 2014; Zeineldin et al., 2016), or it was performed on one side of the thorax exclusively (Abutarbush et al., 2012). As a matter of fact, bovine respiratory disease affects principally the cranio-ventral areas of the lung (Panciera and Confer, 2010). Moreover, the present study, in agreement with previous ones, found just a moderate agreement between the right and the left side of the thorax (Buczinski et al., 2014; Rademacher et al., 2014). Hence, excluding cranial lobes from the ultrasonography examination or evaluating only one side of the thorax could lead to a consistent loss of information and result misinterpretation.

As previously reported, no correlation was found between the presence of TU lung consolidation and the presence of comet tail artifacts/B-lines (Buczinski et al., 2014). The presence of at least three comet tail artifacts in the same site is considered a pathological finding, having been related to alveolar-interstitial syndrome in humans , or cardiogenic pulmonary edema in canine species (Zhang et al., 2006; Isciandro et al., 2014; Ward et al., 2017). The results of the present study are in accordance with previous reports in bovine practice, with comet tail artifacts present in almost all dairy cows and calves, regardless of their health condition, and with less frequency in beef calves (Scott, 2012; Buczinski et al., 2014; Rademacher et al., 2014). Nevertheless, their presence was correlated with viral or bacterial infection, but interestingly not always with associated lung consolidation (Ollivett et al., 2015; Ollivett and Buczinski, 2016). In human medicine, comet tail artifacts are correlated with interstitial or alveolar edema, lesions which may usually be found in the first stages of BRD (Zhang et al., 2006; Panciera and Confer, 2010). In the present study, 2 calves with pathogenic bacteria identified from lower respiratory tract samples, had only comet tail artifacts as lung lesions, but they developed lung consolidation afterwards. Further studies regarding the formation, development and importance of comet tail artifacts in BRD, for instance by analyzing their

number and structure per probing site, could evaluate whether they might be a predictor marker of further lung consolidation and contribute to early diagnosis of respiratory diseases in cattle.

The accuracy of clinical examination on Piedmontese calves, compared to thoracic ultrasonography, showed a higher accuracy when compared with previous studies conducted on veal and dairy calves (Buczinski et al., 2014, 2015). In the aforementioned ones, the clinical examination was performed by means of a standardized scoring systems, the Calf Respiratory Scoring Chart (CRSC) of the University of Wisconsin (McGuirk, 2008), which assigned a score from 0 to 3 to five different clinical signs (body temperature, nasal discharge, cough, ocular discharge and ear position), depending on their gravity. The final score ranged from 0 to 12 and the animals with an overall score > 4 are considered positive for BRD. Using this scoring chart, the presence of only one clinical signs, even if with the higher degree of severity, did not result in the identification of the animal as BRD-affected. Moreover, the authors did not included auscultation in the clinical examination when the accuracy was evaluated. This confirms the importance of an accurate physical examination - including auscultation - in order to increase the accuracy of clinical examination. Thoracic examination showed also a higher accuracy than clinical examination in identification of calves carrying pathogenic bacteria at lung level. Similar results were found by Ollivett et al. (2015), who found thoracic ultrasonography consolidation attributable to bacterial infection in apparently healthy calves.

Bacterial culture

On the day of inclusion, pathogenic bacteria were found in TTA fluid of both calves with or without lung consolidation. Previous studies reported the findings of pathogenic bacteria in TTA of clinically healthy calves, concluding that this pathogenic bacteria could be also found in lungs of healthy animals (Angen et al., 2009). However, in the present study, all calves with initial pathogen presence developed lung lesions during the study period. As previously mentioned, since thoracic ultrasonography allows higher differentiation between animals with or without pathogens in the lower respiratory tract than clinical examination (Van Driessche et al., 2017), it is consequently possible that pathogens identified by mean of bacterial culture were the ones which eventually caused the further development of lung lesions. However, it could be also possible that the lung lesions developed as a result of another infection or, if they had already been present on the day of inclusion, ultrasonography may have failed to detect them. Nevertheless, considering the high accuracy reported for ultrasonography, when compared with post-mortem examination (Rabeling et al., 1998; Ollivett et al., 2015), this last hypothesis did not entirely explained the present results. It is also worth noting that development of lung lesions occurs rapidly after experimental infection, and could be easily identified by ultrasonography (Breider et al., 1988; Reinhold et al., 2002; Lubbers et al., 2007; Ollivett et al., 2013). An artificially induced infection, with standardized bacterial dose, could not be

representative of a natural one. In fact, clinical signs or lung consolidation deriving from the latter, depend on a variety of factors, such as diversity and concentration of involved bacterial species and host immunity response, which are variable and cannot be estimated *a priori* (Panciera and Confer, 2010). It can be hypothesized that in the case of a natural infection, the development of lung consolidation could require more time, compared with experimental infection. An additional sampling of the examined calves at each experimental time would have likely provided more information, but the authors decided to avoid it, in order to comply with animal welfare. The results of the present study confirm what recently stated by Timsit et al. (2017b): it is premature to affirm that in healthy animals, bacterial pathogens could be inhabitant of the lower respiratory tract by considering the sole result of bacterial culture along with posing a distinction over healthy and ill subjects only considering clinical signs. Besides that, in animals who had developed them, pulmonary lesions persisted during the whole study period. Indeed, since in experimentally infected calves lung consolidation was ascertained at post-mortem examination, which took place from 1 to 3 weeks after the initial bacteria inoculation (Mathy et al., 2002; Hermeyer et al., 2012), it is not unlikely that pulmonary lesions detected in this project during the follow-ups may have been caused by the bacteria found in the TTA fluid on the day of inclusion. Moreover, Mycoplasma genus was identified in more than 50% of TTA samples and most of them contained *M. bovis*. *Mycoplasma bovis* has been reported to induce the formation of chronic pulmonary lesions, which eventually failed to respond to medical treatment; this might explain the persistence of lesions in those subjects who developed them, despite the antimicrobial treatment (Caswell et al., 2010).

Genetic analysis

Metabarcoding is a promising technique for identifying microbial communities and new bacterial species, whose isolation and culture from collected sample is difficult to achieve (Taberlet et al., 2012). In fact, it led to the identification of several Operational Taxonomic Units (OTUs), even in samples where no bacteria were identified by mean of traditional culture methods.

At the time in which the study was performed, there were scant data concerning the bovine respiratory microbiota. Hence, to the authors' knowledge, this is among the first studies comparing the microbiota of lower respiratory tract of calves with or without lung consolidation, sampled by mean of trans-tracheal aspiration. Recent studies have highlighted the presence of several bacterial communities in the bovine nasopharynx, thus assuming that potential pathogenic bacteria are common inhabitants of the upper respiratory tract in healthy cattle (Holman et al., 2015b; a, 2017; Lima et al., 2016; Timsit et al., 2016, 2017a; Gaeta et al., 2017). The results of the present study are consistent with previous findings, notably regarding the high number of identified phyla ,and those which appeared to be dominant in the bovine upper respiratory tract microbiota: Proteobacteria,

Tenericutes, Firmicutes, Actinobacteria, and Bacteroidetes (Holman et al., 2015b, 2017; Lima et al., 2016; Timsit et al., 2016b, 2017; Gaeta et al., 2017; Zeineldin et al., 2017a; b).

Moreover, data obtained in the present study showed that microbiota of the upper respiratory tract presented some differences, depending on the animals' farm of origin. However, the number of calves was not equally distributed among the farms, and this difference was statistically significant based only on the unweighted UniFrac distance, which accounts for the presence/absence of observed OTUs. While, it is difficult to draw general conclusions from the present data and further studies are needed to confirm this difference, it is reasonable to assume that environmental factors can influence, at least partially, nasal microbiota composition.

In fact, variations in microbiota composition of the upper respiratory tract of calves following relocation to a new environment, have already been documented (Timsit et al., 2016b; Holman et al., 2017). Nevertheless, several factors have been observed to influence the development and composition of nasopharyngeal microbiota in humans (e.g. mode of birth delivery, breastfeeding), possibly predisposing to the development of respiratory diseases (Biesbroek et al., 2014a; Bosch et al., 2016). Moreover, differences in microbiota composition in relation to geographical provenience have also been found in humans (Stressmann et al., 2011).

Four of the five most abundant phyla identified in the lower respiratory tract were the same as those found in the upper respiratory tract (Proteobacteria, Tenericutes, Actinobacteria, and Bacteroidetes), albeit with different abundance, though Fusobacteria seemed to be characteristic of this ecosystem. Similar results were reported by Zieneldin et al. (2017b). Identification of phylum Fusobacteria in the bovine upper respiratory tract has rarely been reported and each time with low abundance (Holman et al., 2015b; Lima et al., 2016; Gaeta et al., 2017; Zeineldin et al., 2017b). In other species, such as the dog and humans, Fusobacteria is one of the most abundant phyla found in the oral cavity, but it is less abundant in the nasopharynx (Sturgeon et al., 2013; de Steenhuijsen Piters et al., 2015; Ericsson et al., 2016). Furthermore, in the human lung, the abundance of Fusobacteria varies in relation to its presence in the oral cavity, leading to the hypothesis that the oral microbial community could be a bacterial source for lung microbiota composition (Erb-Downward et al., 2011; Bassis et al., 2015b; de Steenhuijsen Piters et al., 2015; Einarsson et al., 2016; Lee et al., 2016).

Overall, the most abundant taxa identified was *Mycoplasma*, belonging to the phylum Tenericutes, which was abundantly found in all NS and TTA samples, regardless of the presence of lung consolidation or bacterial culture results. It was also the most abundant genus identified at bacterial culture. This genus had been previously identified with high abundance in both upper and lower respiratory airways of both healthy and BRD-affected calves (Lima et al., 2016; Timsit et al., 2016; Johnston et al., 2017; Zeineldin et al., 2017a; b). *Mycoplasma* is spread worldwide and it has frequently been isolated also by mean of bacterial culture and PCR detection from lower respiratory tract samples in calves, regardless of their clinical status (Allen et al., 1991; Angen et al., 2009).

Moraxella, the second most abundant genus identified in the NS samples, has been reported by Timsit et al. (2016), Lima et al. (2016) and Zeineldin et al. (2017a). Lima et al. (2016) also found a correlation between the presence of *Moraxella* and the development of BRD. In cattle, *Moraxella bovis* is the etiological agent of Infectious Bovine Keratoconjunctivitis (IBK), but *Moraxella* genus has rarely been isolated in course of BRD (Catry et al., 2007; Angelos, 2015). In children, the role of this genus in the development of respiratory disease is controversial and is probably correlated with the identified species. In fact, it has been associated with the development of asthma and respiratory disease, but also with a more stable microbiota, which is linked to a lower risk of developing respiratory disease (Biesbroek et al., 2014b; Sakwinska et al., 2014; Depner et al., 2017). *Corynebacterium* was identified in all NS samples, and previous studies have reported its relatively high abundance in the bovine upper respiratory tract (Holman et al., 2015b; Lima et al., 2016; Timsit et al., 2016). In children it has been correlated with a reduced risk of developing otitis media and respiratory disease (Laufer et al., 2011; Bosch et al., 2016).

The second most abundant genus identified in the TTA samples, and the main component of the phylum Proteobacteria, was *Pasteurella.* It was comprised mostly of *P. multocida* and it was also identified with a lower abundance in the NS samples. By contrast, in dog, *Pasteurellaceae* were more abundant in oral than in lung and nasal samples, and in sheep, a higher abundance of *Pasteurellales* were found in the throat (Ericsson et al., 2016; Glendinning et al., 2016).

A possible constraint to the present study might have been the absence of a blank control and a mock community, in order to calculate the PCR error rate and to evaluate the possible presence of contaminants in the DNA treatment process. At the time of this study, inclusion of sequencing controls was not a widespread practice, and was not comprised in similar studies (Holman et al., 2015b, 2017; Timsit et al., 2016).

Forty-five out of the 305 identified genera were possible contaminants, according to Salter et al. (2014), but their relative abundance was low (Appendix 1: Table S1). Three of these genera were found with an average relative abundance higher than 1% only in the NS samples: Sphingomonas, Psychrobacter, and Corynebacterium. The overall relative abundance of contaminant genera was 0.6% in the TTA and 11.35% in the NS samples. As suggested by Salter et al. (2014), since contaminant issues are more relevant in low biomass samples, it could be hypothesized that the bacterial load in the NS samples was lower, which would be odd, given that the upper airways are probably far more exposed to a higher environmental bacterial load (Ericsson et al., 2016). A more probable explanation could be that these genera were more abundant in the NS samples because of environmental contamination of the upper respiratory tract, rather than contamination occurred during the samples' analysis due to low biomass.

Comparison of TTA fluid and NS samples bacterial communities identified by metabarcoding

Alpha and beta diversity account for the diversity within samples (richness and evenness) and between samples, respectively (Di Bella et al., 2013). The results of the present study showed significant differences in alpha diversity and beta diversity between the upper and the lower respiratory tract microbiota. In other words, the number of species composing the lower respiratory tract microbiota was lower and these species differed by abundance and type from those of the upper respiratory tract microbiota. Zeineldin et al. (2017b) also found difference in beta diversity, but not in alpha diversity, by comparing the microbiota composition of upper and lower respiratory tract of healthy calves. It was used a different sampling approach (deep nasopharyngeal swab and bronchoalveolar lavage), which could have been influenced by the number of species found in the sample type, while at the same time it did not alter the results obtained for the beta diversity, which measures the variation of taxa abundance between samples. A similar outcome was obtained from studies in humans and dogs (Charlson et al., 2011; Morris et al., 2013; Bassis et al., 2015b; Marsh et al., 2016). In the former, the lung microbiota significantly differed from the oral and the nasal microbiota (Morris et al., 2013; Marsh et al., 2016). In healthy individuals, the lung microbiota partly overlapped the oral one (Morris et al., 2013; Marsh et al., 2016), which is the main source of the composition of the lung microbiota, due to the constant flow of saliva from the mouth, while much less liquid flows from the nose (Bassis et al., 2015b). In upper respiratory tract disease, there is an increased liquid flow from the nose, with a higher potential to consequently affect the lung microbiota (Bassis et al., 2015b). Nevertheless, the disproportion between lung and mouth OTUs presence suggested that the composition of the lung microbiota is not influenced by the oral only, but it may be explained by the proliferation of organisms in the lung environment (Morris et al., 2013).

Analysis of respiratory tract samples obtained from the dog led to the hypothesis of a self-sustaining lung microbiota also in this species, which proved to be considerably more homogeneous than the composition of the oral or nasal microbiota (Ericsson et al., 2016). Moreover, a similar difference, with the upper respiratory tract characterized by an higher richness of species and by different populations, compared with the lower one, has been highlighted also in horses (Bassis et al., 2015a). In the present study, the trans-tracheal approach was used for the collection of samples from the lower respiratory tract, in order to minimize the contamination from the upper respiratory ways. This method has been recommended as optimal for evaluation of the microbiological status of the lower respiratory tract of calves (Angen et al., 2009). The identification of 16 genera in the lower respiratory tract, albeit few and with low abundance, suggests that the lung could be colonized by specific bacterial species also in cattle. Unfortunately, because of the lack of information about the oral cavity microbiota of cattle, no conclusions can be drawn.

The comparison of microbiota by farm of origin showed a statistical difference in its composition only for the NS samples. This suggests that the lower respiratory tract microbiota may be more

homogeneous and resilient than that of the upper respiratory tract, in agreement with results found in the dog (Ericsson et al., 2016). Moreover, these findings account for the hypothesis of a self-sustaining lower respiratory tract microbiota also in cattle.

Characteristics of the lower respiratory tract microbiota based on presence/absence of lung consolidation

The characterization of microbiota based on ultrasonographic lesions allowed to state that bacterial populations are present in healthy lungs. To the author's knowledge, to date only one study compared the microbiota of healthy and consolidated lungs with sampled taken at post-mortem examination (Johnston et al., 2017).

Even though some taxa were more represented in calves with lung consolidation, overall beta diversity did not differ, based on presence/absence of lung consolidation. This suggests that what had been previously assumed in human medicine could also be applied for bovine practice: healthy lungs are normally colonized by a group of bacterial communities, and lung lesions are likely correlated with a modification of this microbiota, in which certain species become more represented (Dickson et al., 2014a). It is not unlikely to suppose that including a higher number of calves would have led to significant differences based on presence of lung consolidation.

The genus *Mycoplasma,* most abundant in TTA samples, did not differ between calves with or without lung consolidation. Thus, some mycoplasma species might be considered as normal inhabitant of the lung microbiota, while others can have a pathogenic effect. Unfortunately, the scarcity of information about *Mycoplasma* species in the TTA samples precluded hypothesis formulation about their pathogenetic role in the present study. Although *M. bovis* is recognized as a major etiologic agent in BRD and otitis media, less is known about other *Mycoplasma* spp., such as *M. dispar* and *M. bovirhinis,* which have been detected less frequently in the bovine respiratory tract (Allen et al., 1991; Thomas et al., 2002a; Ayling et al., 2004; Nikunen et al., 2007b; Angen et al., 2009; Bertone et al., 2015). However, Timsit et al. (2016), reported a high presence of *M. dispar* and *M. bovirhinis* by means of metabacoding in the upper respiratory tract of healthy calves, suggesting they are non-pathogenic commensals. Furthermore, It has been suggested that the lack of isolation with culture methods of these two species could be correlated with an inhibitory effect of other mycoplasmas (Allen et al., 1991, 1992). While it was not possible to investigate more thoroughly *Mycoplasma* genus in order to better characterize the whole species ensemble, bacterial culture suggested the potential pathogenicity of *Mycoplasma bovirhinis,* which was isolated alone in two TTA samples from calves with lung consolidation. Nevertheless, further discriminatory analyses within this genus could provide clues to determine the relationship between these species and their role in BRD.

Moreover, *Ureaplasma,* another genus belonging to the phylum Tenericutes, was more represented in calves with lung consolidation. Even if less critical than *Mycoplasma*, some reports about the identification of this genus in pneumonic lungs are present (Thomas et al., 2002a; Autio et al., 2007). Consequently, it could possibly have a secondary role in the development of the disease.

Other OTUs composing the core microbiota of calves with lung consolidation, and that were more abundant in that group, belong to the family of *Pasteurellaceae,* including *Pasteurella multocida* and *Mannheimia*. The differences in the abundance of this family based on presence of lung consolidation are in line with the results obtained by Johnston et al. (2017). *P. multocida* has been recognized as a common inhabitant of the upper respiratory tract and considered a potential contributor toBRD, alone or in association with other pathological agents (Autio et al., 2007; Nikunen et al., 2007a; Angen et al., 2009; Panciera and Confer, 2010). *Mannheimia* genus consists of 5 species: *Mannheimia haemolytica, Mannheimia granulomatis, Mannheimia glucosida, Mannheimia ruminalis*, and *Mannheimia varigena* (Griffin et al., 2010). All of these species, except *M. ruminalis*, have been associated with pneumonia in ruminants or others domestic species (Angen et al., 2002). In particular, the most common pathogen involved in BRD is *M. haemolytica* (Griffin et al., 2010). This species, as well as *P. multocida*, has been recognized as a commensal bacterium of the upper respiratory tract, which can also colonize the lower respiratory tract, in presence of predisposing factors (Griffin et al., 2010). The limitation of the present study in identifying the OTUs at species level limited the study of the genus *Mannheimia* but confirmed his role in the pathogenesis of BRD.

A relatively new family correlated with BRD was *Leptotrichiaceae.* Johnston et al. (2017) found this family only in consolidated lung, correlating its presence with the respiratory disease. The present study is consistent with these finding, since this family was found with higher abundance in calves with lung consolidation.

In human medicine, members of this family have been found in the respiratory tract by metabarcoding, and they have been correlated with pneumonia, as well as found commensal in the oropharynx (Kawanami et al., 2009; de Steenhuijsen Piters et al., 2015). In the present study, this family was found likewise in animals without lung lesions, although with a lower abundance. However, as reported by Johnston et al. (2017), these findings are not enough to consider members of *Leptotrichiaceae* as potentially pathogens, but surely their excessive proliferation could be correlated with bovine respiratory disease.

According with Johnston et al. (2017), other two genera were found with a highly abundance in calves with lung consolidation: *Fusobacterium* and *Bacteroides.* Both are not primary lung pathogens, but they have already been identified in consolidated lungs and therefore considered as probable opportunistic pathogens, grown secondarily to a variety of predisposing factors, including the intervention of other bacterial agents (Chirino-Trejo and Prescott, 1983).

Among all, only one taxa tended to be more abundant in calves without lung consolidation: family *Microbacteriaceae.* This family was also found with an abundance higher than 1% in NS samples, confirming the results of previous studies in which members of this family had been recognized as part of the nasopharyngeal microbiota of beef calves (Holman et al., 2017; Zeineldin et al., 2017b). Several genera and species belonging to the family have been identified in soil, urine, human blood, cow feces, cow teat skin and cow milk, though with low abundance (Lin et al., 2004; Delbès et al., 2007; Vacheyrou et al., 2011; Verdier-Metz et al., 2012). In healthy humans, the upper respiratory tract microbiota seems to be the main source for the composition of the lung microbiota (Man et al., 2017). This hypothesis could be transposed to the bovine species as well, since the presence of this family could be reconducted to its high concentration in the nasopharyngeal microbiota. However, in other recent studies where characterization of lung microbiota had been performed, family *Microbacteriaceae* was not highly represented (Johnston et al., 2017; Zeineldin et al., 2017b). These mixed reports might have been influenced by different factors, such as age, breed, management and environment. Surveys carried out in human patients have referred that geographical provenience, mode of delivery and infant feeding could influence the composition of the respiratory tract microbiota (Stressmann et al., 2011; Unger and Bogaert, 2017). In the present study all the calves, except one, were sampled few days after the weaning and they all came from cow-calf operations in which the calf suckled from the dam's teat. The presence of *Microbacteriaceae* on cow teat skin could explain its presence also in lower respiratory tract microbiota of calves included in the present study (Verdier-Metz et al., 2012). Nevertheless, from the results of the present study, an assessment of the role of this family in bovine respiratory health is not viable, and further studies will be required.

Further research is needed in order to evaluate the possible effect of selected management practices, as well as age and breed factors on respiratory microbiota. In fact, even if NGS technology is still considerably limited in veterinary practice due to its high cost, it could be a convenient tool to better analyze BRD pathogenesis and to develop control methods, aimed to antimicrobial usage reduction.

Conclusions

In conclusion, thoracic ultrasonography in beef calves proved to be a quick, convenient and more accurate method for respiratory disease identification, compared to clinical examination. The results obtained from the NS samples suggest that environmental factors (farm, management) may influence the microbial composition of the bovine upper respiratory tract. Moreover, the present findings demonstrate the presence of bacterial communities in the lower respiratory tract in both calves with or without lung consolidation, which significantly differed from those present in the upper respiratory tract. Despite these differences, the composition of the lower respiratory tract microbiota

seemed to be influenced by the one of the upper respiratory tract, although the possible influence of the oral cavity microbiota is still to be investigated. Further studies including characterization of the oral cavity microbiota are needed to confirm this hypothesis. Moreover, the etiopathogenesis of BRD could be explained as a shift of bacterial communities already presents at pulmonary level, rather than an overgrowth of a single pathogenic agent. Further studies are needed in order to evaluate the possibility of developing preventive measures for BRD, based on the shaping of respiratory microbiota from early life.

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SECONDARY PROJECT 1: EVALUATION OF POSSIBLE PREDISPOSING FACTORS AND BIOCHEMICAL PREDICTORS FOR BRD TREATMENT IN FIRST DAYS ON FEED (60 DAYS) IN NORTHWESTERN ITALY BEEF CALVES FATTENING OPERATIONS

BACKGROUND

Bovine Respiratory Disease (BRD) is one of the main health issues in beef fattening operations (Edwards, 2010). It is a multifactorial disease, where stress factors predispose the colonization of the lower respiratory tract by pathogens which are normally inhabitants of the upper respiratory tract (Griffin et al., 2010). Factors associated with the animals, such as weight, gender or breed may influence BRD development (Taylor et al., 2010). Lighter-weight calves are suggested to have higher risk of developing BRD (Sanderson et al., 2008; Hay et al., 2017) and females are reported to have a lesser risk for BRD morbidity and mortality (Muggli-Cockett et al., 1992; Snowder et al., 2006).

The calves reared in beef fattening operations are also subject to a number of stress factors, mostly in the first days on feed, a period characterized by the highest BRD incidence (Snowder et al., 2006). These factors include weaning, transportation and commingling (Taylor et al., 2010). Both abrupt weaning and transportation stress are reported to induce an increment in cortisol and catecholamines concentrations, with a possible consequent impairment of immune defense (Odore et al., 2004; Burdick et al., 2011; Kim et al., 2011). Moreover, commingling is not only a source of stress, but it can increase the opportunity for naïve calves to be exposed to pathogens (Sanderson et al., 2008). Among the predisposing factors for BRD morbidity, virus infections assume an important role, as their presence is more frequently recognized as preliminary to bacterial respiratory tract colonization (Panciera and Confer, 2010). The most frequently identified viruses in BRD are: Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 virus (PI3V), Bovine Herpesvirus type 1 (BHV-1) and Bovine Viral Diarrhea Virus (BVDV) (Panciera and Confer, 2010). Moreover, even if seroconversion during the fattening period was correlated with increased BRD incidence (Fulton et al., 2000), animals whose seropositivity had been confirmed prior to arrival showed lower risk to develop BRD in Australian and Canadian feedlots (Connor et al., 2001; Hay et al., 2016).

Acute phase proteins (APP) are non-specific markers of tissue damage, and can increase following inflammation, infections or stressful events (Ceciliani et al., 2012). They increase in course of BRD, regardless the severity of clinical signs, but also following transport or abrupt weaning (Gånheim et al., 2007; Giannetto et al., 2011; Idoate et al., 2015). Among them, haptoglobin seemed to be more specific for the identification of affected animals, because it is less sensitive to stress factors and a better indicator for the gravity of the inflammation, compared to other acute phase proteins (Carter et al., 2002; Humblet et al., 2004; Gånheim et al., 2007).

Another consequence of transportation stress is the oxidative stress (Chirase et al., 2004), namely the result of an increased reactive oxygen metabolites (ROM) production, which exceeds the capacity of antioxidant system compensation (Miller et al., 1993). ROMs are commonly involved in physiological processes and are also endowed with bactericidal properties but, when their concentration is excessive, they may become harmful to the same organism that had them synthesized (Miller et al., 1993; Brieger et al., 2012). As a matter of fact, they are highly reactive molecules that, at high levels, can react with lipid, proteins and carbohydrates, thus inducing cellular functional alterations or even necrosis, with consequent tissue damages, metabolic alterations or impairment of immune defenses (Miller et al., 1993; Brieger et al., 2012). Oxidative stress after transportation had already been demonstrated, through the evaluation of antioxidant potential and lipid peroxidation products (Chirase et al., 2004). These authors found an association between higher concentrations of stress biomarkers and further treatment or mortality caused by BRD (Chirase et al., 2004). However, to the author's knowledge, the concentration of ROM as predictors for BRD treatment has not yet been evaluated. The objective of the present study was to evaluate factors predisposing BRD treatment in the first part of the fattening period (60 days) and the use of haptoglobin and ROM concentration as possible predictors of treatment.

MATERIALS AND METHODS

Farm recruitment

The study was performed in collaboration with the Association for Zootechnical and Agricultural Service (ASAZ), an institution accredited by Piedmontese region that provides zootechnical consultancies as part of the Piedmontese Rural Development Program 2014/2020. The association is composed by six veterinary practitioners, working in bovine practice. The farms, being part of the clientele of these practitioners, are beef cattle fattening or veal calf units.
A meeting of about 2 hours was organized in order to explain the study design and objectives to the farmers. Farms included in the study were fattening operation, located in Piedmont region, that imports beef heifers and bulls, at the age of 8-12 months, from France. At the initial meeting, 45 farmers were present. Among them, 30 met the inclusion criteria and agreed to participate at the study. The 77% of the farm were located in the province of Cuneo, while the other 23 % in the province of Turin.

Study population

The farms were visited at least once, from April to December 2015, in order to examine and sample heifers and bulls arriving from France. At least one incoming batch for farm was randomly selected during the study period. The examination of the batches was performed within three days

following arrival. Farmers were asked to not treat or vaccine the animals before the visit. At least the 20% of the animals were selected from each incoming batch. The number of animals included was calculated in order to obtain an absolute precision of 5%, with a 95% confidence interval, assuming a disease prevalence of 18% (Assié et al., 2009; USDA, 2013). Data about origin, transport duration, average animal weight of the batch, breed and sex were collected, and a physical examination was performed for each animal. Transport duration was obtained by mean of transport official document. The average batch weight was obtained at arrival, when all the batch had been weighted in group while still on the truck. The following clinical signs were considered as indicator for BRD affection: fever (> 39.5°C), tachypnea (respiratory rate > 36 apm), presence of cough, nasal discharge or ocular discharge. The farmers and the private practitioners collaborating were unaware of the results of clinical examination, in order to avoid being influenced on their treatment decisions. After sampling, the animals were vaccinated within 7 days. All farms included at least 3 viral agents (BRSV, BHV-1, BVDV) in the vaccination protocols, while 21 farms included also bacterial agents (*Mannheimia haemolytica* serotype A1). Twenty-one farms administered parasiticides treatment within 7 days post arrival. For each subject, the owner had to sign an informed owner consent to authorize clinical procedures on his/her animals.

Sample collection

Blood samples were collected from coccygeal vein with Vacutainer serum tube and transported to the laboratory of Department of Veterinary Sciences of Turin. Samples were centrifuged at 3600 x g for 5 minutes and serum was collected and stored at -80°C.

Laboratory analysis

Serum samples were analyzed with indirect ELISA for the detection of antibodies against Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza-3 virus (PI3V), Bovine Herpesvirus 1 (BHV1) (SVANOVA Biotech, Uppsala, Sweden) and Bovine Viral Diarrhea Virus (BVDV) non-structural protein 2-3 (IDEXX Laboratories Inc., Maine, USA) by commercially available enzyme-linked immunosorbent assays (ELISA); each assay was performed in accordance with the manufacturer's instructions.
Moreover, haptoglobin (HP) and Reactive Oxygen Metabolites (ROM) concentration was determined with colorimetric methods (Diacron Labs, Grosseto, Italy; Tridelta Development Ltd, Co. Kildare, Ireland) applied on COBAS C501 instrumentation.
All analyses were performed by the laboratory of the *Zooprofilattico Sperimentale delle Venezie, PADUA (Italy)*

Bovine respiratory disease treatment data

The farmers were asked to keep record of BRD treatment performed on sampled calves in the first 60 days on feed, with the relative dates of administration. Only treatments reported for the single animal were considered, excluding prophylactic treatment. Moreover, only the first treatment performed was considered for the analysis, representing the first manifestation of the disease.

Statistical analysis

Statistical analysis was performed by a freeware statistical software package R v.3.4.1. The Shapiro-Wilk normality test was used to determine whether the data followed a normal distribution. Numeric data (average batch weight, transport time, ROM and Hp concentration) were reported as mean and standard deviation (± SD) or as median (min, max), based on data distribution. Average batch weight and transport duration were also reported as categorical variables based on median and 25^{th} and 75^{th} percentile and median value, respectively. Nominal data (breed, sex, weight category, clinical signs, season of sampling, prophylactic/metaphylactic treatment, serology results) were expressed as frequency, percentage, or both.

Univariate analysis of nominal data was performed with contingency table analysis by Fisher's exact test. In addition, for each 2 × 2 contingency table, the odds ratio (OR) and the 95% confidence intervals of the odds ratio (95% OR CI) were calculated. Variables that meet a cut-off of $P \leq 0.20$ at the univariate analysis were entered into a logistic regression model for the multivariate analysis. Two separate logistic regression models, one for treatment within 7 days and one for treatment within 60 days were constructed having treatment as the dependent variables and historical and clinicopathologic data as the independent variables. The most parsimonial final model was selected, via backward elimination, with a Wald P value of 0.05 as the removal threshold, given an acceptable log-likelihood ratio test value. Model fit was evaluated by Pearson's and Hosmer-Lemeshow's goodness-of-fit test. A value of $P > 0.05$ indicates that the data adequately fit the model used. The Wilcoxon rank sum test was used in order to evaluate difference in HP and ROM concentration, based on treatment within 7 and 60 days from arrival. P was set at < 0.05.

RESULTS

Animals data and serological evaluation

Overall, incoming animals were examined and sampled in 24 farms. In 5 farms out of 30, animals had already been treated and/or vaccinated by the receiving farmer, while in one, BRD treatment record in the first 60 days was not available.

Overall, 27 batches were included. Twenty-one farms were visited once, while three were visited twice. The total number of arriving animals was 798, of which 179 (22.4%) were examined and sampled. The median number of arriving animal per batch was 26 (12, 60) and the median of animals sampled from each batch was 6 (4, 12). The two most represented breeds were Blonde d'Aquitaine (44.6%) and Limousine (34.1%) and the median average batch weight was 332 kg (195, 470). The batch collection centers were located in 4 France regions: Auvergne-Rhone-Alpes, Bourgogne-Franche-Comté, Nouvelle Aquitaine and Occitanie. Eighty-nine animals (49.7%) originated from the same regions in which the collection center was located (table 1). No other information regarding the origin or the medical history of the animals were available. The median time of transport was 12 hours (2, 18). No batches stopped in a lairage during the transport. At arrival, the most frequent clinical sign was tachypnea, followed by nasal discharge and most of the animals (80.4%) showed only one or none clinical signs. Sampling was carried out mainly in spring (90.5%). Further details about animal distribution in the considered category are reported in table 8.

Concerning the serology evaluation, most of the animals had antibodies against PI3 (75.4%) and BRSV (63.7%), while antibodies against BHV-1 and BVDV were found in a lower percentage of animals (10% and 5.6%, respectively). Median (min, max) of percentage of positive samples per farm was 80% (20%, 100%), 60% (0%, 100%), 0% (0%, 100%) and 0% (0%, 33%) for PI3, BRSV, BHV-1 and BVDV, respectively.

In the first 60 days on feedlot 35 animals (19.6%) were treated for BRD, of which 17 (48.6%) were treated in the first week and no subjects died during the observation period. The variables that meet the inclusion criteria for the inclusion in the multivariate model are reported in table 9 and 10 for treatment within 7 days and 60 days post arrival, respectively.

Table 8. Animal distribution in the considered category: breed, sex, region of provenience, transport duration ≥ 12 h, average batch weight categories (≤ 275 Kg, 276 – 332 Kg, 333 – 404 Kg, ≥ 405 Kg), season of sampling, type and number of clinical signs and prophylactic/metaphylactic treatment at arrival. NA = not available; M = male; F = female, RR = Respiratory rate. The others category of the region of provenience, included: Bretagne (n=3), Pays de la Loire (n=1), Centre-Val de Loire (n=7), Hauts de France (n=2) and Grand est (n=6).

Parameter	Category	Number of animals	Percent
Breed	Blonde d'Aquitaine	80	44.6%
	Limousine	61	34.1%
	Charolaise	15	8.4%
	Mixed breed	23	12.9%
Sex	M	156	87.2%
	F	23	12.8%
Average batch weight	≤ 275 Kg	53	29.6%
	276 – 332 Kg	39	21.8%
	333 – 404 Kg	39	21.8%
	≥ 405 Kg	48	26.8%
Region of provenience	Nouvelle Aquitaine	44	28.5%
	Occitanie	50	27.9%
	Auvergne Rhone Alpes	47	26.3%
	Bourgogne-Franche-Comté	12	6.7%
	Others	26	10.6%
Transport duration ≥ 12 h	Yes	110	61.4%
	No	69	38.6%
Season of sampling	Spring/summer	162	90.5%
	Autumn/winter	17	9.5%
Cough	Yes	21	11.7%
	No	158	88.3%
Fever (> 39.5°C)	Yes	25	14%
	No	154	86%
Nasal discharge	Yes	42	23.5%
	No	137	76.5%
Ocular discharge	Yes	9	5.1%
	No	170	94.9%
Tachypnea (RR > 36 apm)	Yes	55	30.7%
	No	124	69.3%
Number of clinical signs present	0	79	44.1%
	1	65	36.3%
	2	26	14.5%
	≥ 3	9	5.1%
Prophylactic/metaphylactic treatment	Yes	89	49.7%
	No	90	50.3%

Table 9: Univariate analysis results were reported for variables included in the multivariable model (P ≤ 0.2) assessing predisposing factors for treatment within 7 days post arrival for 179 beef calves imported in northwestern Italy fattening operation. OR = Odds Ratio; CI = Confidence interval.

Parameter	OR	95% CI	*P* value
Limousine breed	0.1	0.003 - 0.7	0.01
Mixed breed	3.3	0.8 - 11.6	0.05
Transportation duration ≥ 12 hours	2.2	0.6 - 9.5	0.2
Arrival weight between 276 and 332 Kg	0.4	0 - 0.8	0.03
Arrival weight ≥ 405 Kg	2.1	0.6 - 6.4	0.2
Autumn/winter season	3.8	0.8 - 15.1	0.05

*Table 10. Univariate analysis results were reported for variables included in the multivariable model (P ≤ 0.2) assessing predisposing factors for treatment within 60 days post arrival for 179 beef calves imported in northwestern Italy fattening operation. OR = Odds Ratio; CI = Confidence interval. *BRSV, BHV1, BVDV, PI3*

Parameter	OR	95% CI	*P* value
Limousine breed	0.3	0.1, 0.9	0.02
Blonde D'Acquitaine breed	2.2	1, 5	0.06
Males calves	0.3	0.1, 0.9	0.02
Transportation duration ≥ 12 hours	2.5	1, 6.7	0.04
Arrival weight between 276 and 332 Kg	0.4	0.1, 1.3	0.1
Autumn/winter season	2.8	0.8, 9.2	0.09
Presence of antibodies against BVDV	2.9	0.6, 13.3	0.1
Presence of antibodies against BRSV	1.8	0.8, 4.8	0.2
Presence of antibodies against at least 2 viruses	1.8	0.8, 4.3	0.1
Presence of antibodies against all 4 viruses evaluated*	4.3	0.3, 60.7	0.2
Prophylactic/metaphylactic treatment	1.9	0.8, 4.6	0.1

At multivariable analysis two variables were associated with BRD treatment within 7 days: transport duration length ≥ 12 h (OR = 5.2, $P < 0.05$) and autumn/winter season (OR = 8.8, $P < 0.01$). The same variables were associated with treatment within 60 days (transportation duration length ≥ 12 h: OR = 3.6, $P < 0.05$; autumn/winter season: OR = 5.1, $P < 0.05$)

Haptoglobin and Reactive Oxygen Metabolites evaluation

ROM concentration of non-treated animals was higher than that of animals treated both within 7 and 60 days ($P < 0.05$), while HP did not differ between treated and non-treated animals, even considering the 7 or the 60 days period ($P > 0.05$). Median (min, max) HP and ROM concentration for animals treated within 7 and within 60 days are reported in table 11.

Table 11. Values of haptoglobin (HP) and reactive oxygen metabolites (ROM) are reported as median (min, max), based on treatment within 7 or 60 days post arrival. [a,b] indicate data that statistically differed (P < 0.05).

Parameters	Category	ROM (U/carr)	HP (mg/dl)
Treatment within 7 days	Yes	65 (40, 192)[a]	35 (15, 280)
	No	92 (40, 228)[a]	31 (11, 150)
Treatment within 60 days	Yes	73 (40, 192)[b]	33 (11, 280)
	No	96 (40, 228)[b]	30 (11, 150)

DISCUSSION

Consistently with previous findings, the prevalence of BRD reported in the present study was 19.2%, based on treatment records (Snowder et al., 2006; Assié et al., 2009; USDA, 2013). Moreover, about half of the treatment was performed in the first week, and the 97% of the whole treatment was performed in the first 25 days on feedlot, being in line with the common description of BRD epidemiology, which usually has a higher incidence in the first days post arrival (Snowder et al., 2006; Sanderson et al., 2008; Schneider et al., 2009; Panciera and Confer, 2010)

This has been correlated with stressful factors to which animals are submitted at the beginning of the fattening period, such as commingling, handling and transportation (Taylor et al., 2010). The effect of transportation, in particular, has been evaluated in the present study. Transportation has been reported to increase cortisol and catecholamines concentrations, with consequent impairing of the immune system (Odore et al., 2004; Riondato et al., 2008). Some authors correlated the transport-related stress to the handling and loading operations, reporting a negative correlation between the transport duration and the BRD morbidity (Cole et al., 1988; Taylor et al., 2010). However, Pinchak et al. (2004) and Hay et al. (2014), in agreement with the results of the present study, reported that the factor influencing further BRD development was the duration of transportation. In fact, during transportation, animals are subjected to water and food deprivation, which was suggested to be the principal cause of stress during transport (Marques et al., 2012).

Another significant stress factor was the season of arrival, which was associated with an increase rate of treatment in calves during the first days of fattening period. Although interesting, this data should be carefully interpreted, considering the irregular distribution of arrivals throughout the year. Nevertheless, higher incidence of BRD in autumn or winter seasons has been previously reported by several authors (MacVean et al., 1986; Alexander et al., 1989; Ribble et al., 1995; Hay et al., 2017). Notably, autumn and winter are usually characterized by rapid changes in temperature and adverse weather conditions, which could be a potential stress factor for cattle, especially if correlated with other stressful events (transportation, commingling) (Cernicchiaro et al., 2012).

Breed did not show to be a predisposing factor in the present study. Despite previous study reported a correlation between breed type and BRD development, the present study included less breed type, compared to the aforementioned one (Muggli-Cockett et al., 1992; Snowder et al., 2005, 2006; Hägglund et al., 2007). Moreover, none of the breed included in the present study has been reported as highly susceptible (Snowder et al., 2005, 2006).

The gender distribution of the present study was similar to those already reported by other research (Schneider et al., 2009; Hay et al., 2014). It is also worth noting that females have been previously reported to be less at risk of developing BRD (Muggli-Cockett et al., 1992; Snowder et al., 2006; Hay et al., 2017). It was assumed that this difference can be correlated with the fact that many calves arrive at the feedlot as bulls and must be castrated (Taylor et al., 2010). Since castration is indeed a painful and stressful event, it has been hypothesized that this can increase predisposition for the development of BRD (Taylor et al., 2010). In fact, bulls castrated after arrival showed a higher predisposition for BRD, compared with steers castrated shortly before shipping (Pinchak et al., 2004). In the present study, the animals were not castrated, neither before nor after shipping, thus they were not affected by further stressful events, and this could account for the lack of difference between males and females. However, since the distribution of animals based on gender was not homogeneous, this could have influenced its evaluation as influencing factor. Hay et al. (2017), found that sex had moderately estimated population-level effects, but the 95% credible interval included negative values, therefore they considered this estimate too imperfect to drawn conclusion.

In the present study, the weight at arrival was not detected as a predisposing factor for treatment in the first 60 days post arrival. This is in contrast with the assumption that lighter calves have higher predisposition to develop BRD, as suggested in several previous studies (Gummow and Mapham, 2000; Sanderson et al., 2008; Taylor et al., 2010; Hay et al., 2017). On the other hand, it has to be considered that the above studies reported very different interval of weight at arrival, with as many different cut-offs point for the analysis. Gummow and Mapham (2000), for instance, based their analysis by dividing the group into two classes, using a cut-off of 245 Kg, while Hay et al. (2017), made 4 categories, with the lighter beneath 400 kg. The categorization chosen in the present study differed from the aforementioned ones and this may explain the difference in the obtained results.

Moreover, a difference in BRD incidence based on weight was not unanimously reported. In fact, surveys reporting arrival weight range akin to the one of the present study, did not evidence a concurrent influence on subsequent development of BRD (Alexander et al., 1989; Gardner et al., 1999).

According to our findings, the region of origin was not correlated with treatment. However, an earlier survey carried out in France, showed a geographical distribution of BRD incidence in selected French departments (Gay and Barnouin, 2009). In the present study, the analysis was based on region of origin, in order to avoid having excessively small groups for the analysis. The main animal-exporting regions included in this project had a heterogeneous distribution of BRD incidence, based on Gay and Barnouin (2009) report. However, they did not describe a production-type distribution, therefore no information are provided regarding the specific category of interest in the present research (Gay and Barnouin, 2009).

The presence of clinical signs at arrival was not correlated with subsequent treatment for BRD. It has to be considered that most of the animals had one or no clinical signs, the most frequently identified being tachypnea, which is not a specific sign of respiratory disease, as respiratory rate may increase also following a stressful event (Burdick et al., 2011; Wolfger et al., 2015). Body temperature cannot be considered a specific sign of BRD neither, considering that it usually increases during transportation, accordingly to animal temperament (Burdick et al., 2010). Moreover, the body temperature cut-off for the diagnosis of BRD has proved to be variable among literature (from 39.4°C to 40.6°C). Consequently, the selected cut-off in the present study, especially considering the effect that transportation could exert over body temperature, may have led to a misclassification of healthy and BRD-affected calves. Furthermore, it has largely demonstrated that clinical examination has not only low accuracy, but it can also vary among different operators (Buczinski et al., 2016b; Timsit et al., 2016a). This can explain why no correlations have been found between the presence of clinical signs at arrival, identified by the authors, and the subsequent treatment, performed by the farmers or the practitioners.

High seroprevalence of BRSV and PI3 antibodies found in the present study reflects the results reported by Hay et al. (2016) and Ferella et al. (2017) in Australian and Argentinian feedlot cattle, respectively. Moreover, in a Canadian study, similarly high seroprevalence was reported for PI3 in calves at arrival, while the percentage of animals seropositives for BRSV was less than the one found in the present study (Durham et al., 1991). Seroprevalence reported by Hay et al. (2016) and by Durham et al. (1991) for BVDV and BHV-1 were higher than the one reported in the present study. This was probably due to eradication plans performed in all France, in order to reduce the prevalence of these two diseases. The France BHV1 eradication plan, started in 2006, is nation-wide and mandatory, imposing to vaccinate the herds with positive animals, but not to the BHV-1 free (Groupements de Défense Sanitaire; Ngwa-mbot et al., 2015). The BVDV eradication plan is

mandatory in certain departments, volunteer and strictly recommended by the *Groupements de Défense Sanitaire* in the rest of France, and it is based on the identification and elimination of immunotolerant persistent infect calves (IPI) and the serology for adult cattle (Contre la bvd). Contrarily to previous studies, that reported a correlation between seropositivity and a reduction risk for BRD, in the present study no associations were found between presence of viral antibodies and BRD treatment (Durham et al., 1991; Booker et al., 1999; Hay et al., 2016). Considering that all farms had a vaccination protocol that including BHV1, BRSV and BVDV, and 17 farms vaccinated for PI3 as well, the correlation between serology results and treatment in the first days post arrival is hardly evaluable. However, given that farmers had scarce information about animal health management before shipping, the obtained results were interesting information for them, showing a not homogeneous seroprevalence within and between batches.

Haptoglobin concentration at arrival did not showed to be a good predictor of BRD treatment in the first days post arrival. Haptoglobin is an acute phase protein, which aspecifically increases in case of inflammation, infection or stress. It has been proven to increase during BRD, although it has been mainly correlated with larger tissue damages, chronic diseases and bacterial infections (Heegaard et al., 2000; Svensson et al., 2007; Orro et al., 2011). Thus, despite its fast augmentation following the infection beginning, its increase coincides with the onset of clinical signs, failing to be a viable predictor value for future development of the disease (Svensson et al., 2007).

On the contrary, the ROM appeared to be lower in animals that were later treated for BRD. ROM are natural byproducts of oxygen metabolism, playing an important role in many physiological processes and in the modulation of the inflammatory response (Brieger et al., 2012). When the ROM production exceeds the antioxidant availability, the result is a condition of oxidative stress (Bernabucci et al., 2005; Brieger et al., 2012). To the author's knowledge, studies evaluating the ROM concentration in beef calves are scant, even if it has already been demonstrated that transportation contributes to a condition of oxidative stress and subsequent arise of oxidative stress biomarkers, which can predispose and be correlated to BRD development (Chirase et al., 2004). Nevertheless, it has been demonstrated that not only an excess of ROM in human patients can be dangerous, but also a too low concentration, reflecting in a reduced antimicrobial defense (Brieger et al., 2012). In fact, mitochondrial respiration is not the only source of ROM, as they can also be synthesized by NADPH oxidase enzymes (NOX) (Bedard et al., 2009). In human patients, the NOX activity resulted influenced by genetic individual variability, which thus influenced the amount of ROM produced (Brieger et al., 2012). Considering the results of the present study, it may be assumed that a similar genetic component is also present in cattle, resulting in animals with lower capacity in ROM production being more susceptible to BRD development. Nevertheless, considering the lack of information about ROM and the absence of cut-off values in bovine species, further studies are needed in order to confirm this hypothesis.

In conclusion, the present study showed that, in northwestern Italian fattening operations, transportation duration and season of shipping of imported animals from France were the two-major factor associated with BRD treatment in the first day post arrival. Moreover, haptoglobin did not result a good predictor for BRD treatment. ROM concentration, on the other hand, may have an interesting application in the prediction of BRD, but further studies are needed to draw conclusions.

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SECONDARY PROJECT 2: MONITORING OF ANTIMICROBIAL DRUG USE AND EVALUATION OF RISK FACTORS ASSOCIATED WITH INCREASE ANTIMICROBIAL USAGE IN NORTHWESTERN ITALY BEEF CALVES FATTENING OPERATIONS

BACKGROUND

Bovine respiratory disease (BRD) is the disease with the highest prevalence in beef cattle industry (USDA, 2013). Besides being a health issue, BRD is an important cause of economic loss for the farmers as well, in terms of reduced average daily gain, calves death and treatment costs (Snowder et al., 2006; Cernicchiaro et al., 2013). To date, the amount of treatments is no longer a mere economic problem for the farmers, but it has raised interest in public health. In fact, many national monitoring programs in Europe reported an increase of antimicrobial resistance in human pathogens, correlated with the usage of certain molecules in food-producing animals (ECDC/EFSA/EMA, 2017). A 2014 review reported an estimation of 700,000 deaths for year attributed to antimicrobial resistance, and the author had estimated 10 million deaths per year in 2050, had the consumption of antimicrobial not been controlled (O'Neill, 2014). Consequently, it is increasingly important to figure out how to reduce antimicrobial usage in livestock. The European Commission published guidelines for antimicrobial usage in veterinary medicine, which promote the prescription of antimicrobial drugs following a susceptibility test, the use of methaphylaxis treatment only when needed and the discontinuation of all type of prophylaxis treatment (European Commission, 2015). Monitoring antimicrobial consumption is the first step to reduce their usage, because it helps to identify situations where antimicrobial administration could be potentially avoided. In Netherlands, as well as in Denmark, Finland, Sweden, United Kingdom, France and Belgium, mandatory or voluntary plans for reduction of antimicrobial usage, most of all the Highest Priority Critically Important Antimicrobials (fluoroquinolones and cephalosporines 3rd and 4th generations), have been implemented, and guidelines regarding a responsible use of antimicrobials have been distributed (EFSA/ECDC/EMA, 2017). These plans included national monitoring of antimicrobial sales and consumption in different production categories (Jensen et al., 2004; MARAN, 2011). The countries that initially enforced these plans have already recorded a considerable reduction in sales of certain antimicrobial, with a consequent reduction of antimicrobial resistance (EFSA/ECDC/EMA, 2017). In Italy, to the author's knowledge, only one study on antimicrobial use monitoring was conducted in the cattle fattening sector in north-eastern Italy (Caucci et al., 2018)**.** The fattening rearing of young bulls and heifers imported from other European countries is an important source of meat production in Italy (Cozzi et al., 2009). Most of the fattening units are located in northern Italy and most of the animals are imported from France and they are housed indoor in multiple pens (Cozzi et al., 2009). Further studies regarding antimicrobial use monitoring are needed in order to have a nation-wide

view of antimicrobial use. Consequently, the present study aimed to monitor the antimicrobial usage in fattening operation units in north-western Italy and to identify possible factors influencing antimicrobial usage.

MATERIALS AND METHODS

Farm recruitment

Data of farm recruited are reported in Secondary Project 1.

Animals and farm data collection

The number of animals imported each year was obtained from the animal stock records, whose farmers are compelled to keep for a period of five years. Moreover, a questionnaire was submitted to the farmers always by the same operator during the study period. The survey included information about: mortality, average weight of the animals at the beginning and at the end of the fattening period, medical history/treatment/vaccinations before arrival, arrival procedures (animals examination, vaccinations, parasiticides administration, prophylactic/metaphylactic treatment, quarantine period, fattening group formation), animals health management (animals daily check, frequency of veterinary visits, confinement of sick animals) and farm characteristics (ventilation, m^2/animal, animals/pen, frequency of straw adding, cleaning and disinfection frequency, depopulation period). A complete list of the information collected is reported in table 12 and 13.

Antimicrobial consumption data

Italian law regarding antimicrobial detention and use in food production animals establishes that every farm hold an official record in which information about animals treatment have to be recorded, including: drug supplier's name and address, package identification number, amount of drug administered in the 24 h, animal identification numbers, date of beginning and end of treatment. These records have to be signed by veterinary practitioners and they are checked at least once a year by public sector veterinarians (art. 79, Italian LD 193/2006).

Farms allowed to have drug stocks also have to keep an additional record on which they note the drugs they purchase and use, including: the day of purchasing/use, the quantity and the numbers, and the identification numbers of animals on which the drugs are used (art. 80, Italian LD 193/2006). These records are checked at least once a year by public sectors veterinarians. In any case, all the farmers are obliged to keep their prescriptions' copy for at least 5 years.

Data about the amount of antimicrobial used and treatment performed during the years 2014 and 2015 were collected from official farmers' records or by prescription bills. Data collected included

the antimicrobial trade name, the pharmaceutical form (oral or parenteral), the pack size (mg for powder and ml for liquids) and the total number of packages used.

Treatment data were collected from the relative official records. These records contain information as: The information obtained from the two types of records allow to classify the antimicrobial drug treatments based on two criteria: oral *vs* parenteral and individual *vs* group. Treatments were identified as “group treatment” when it was reported the identification number of the batch, rather than the one of the individuals. The type of active substance present in each commercial formulation was obtained from its trade name, registered in the official drug handbook of the *Ministero della Salute* (Italian Ministry of Health).

Antimicrobial drug usage was quantified based on the animal daily dose (ADD) methodology (Jensen et al., 2004; Pardon et al., 2012a). The ADD is defined as the average maintenance antimicrobial dose of a drug for the main indication in a specified species (Jensen et al., 2004). ADD values were based on the recommended dose approved by the pharmaceutical companies marketing the drugs in Italy. For ADD the antimicrobial drugs were identified according to the Anatomical Therapeutic Chemical classification system for veterinary medicinal products (ATCvet) (EMA, 2015). When antimicrobials had been registered for different diseases (e.g. respiratory disease and mastitis), the dose selected was the one provided for the more frequent disease in fattening operations (respiratory, gastro-enteric and foot diseases). When the same active substance had different dosage in different commercial products or the recommendations included a range of possible dosage, the mean was used as ADD. The International Units were converted based on converting factors proposed by the European Medicines Agency (EMA) for each active substance (spiramycin 3200 IU/mg; procaine benzylpenicillin 1667 IU/mg, colistin sulphate 20500 IU/mg) (EMA, 2016) For long-acting preparations, the ADD was calculated from the recommended dosage into a 24 h dose, by dividing by long-acting factor (LA factor), defined as the number of days considered under treatment after one application of the drug. The long acting factors were obtained from literature and from the products characteristic released by the pharmaceutical companies (Pardon et al., 2012b; Dedonder et al., 2016; Lava et al., 2016).

The number of ADD (nADD) was calculated with the following equation:

$$a)\quad nADD = \frac{total\ amount\ of\ drug\ administered\ (mg)}{ADD\left(\frac{mg}{kg}\right)\ x\ average\ animal\ weigth\ (kg)}$$

The total amount of drug administered was calculated from drugs records and prescription bills. The average animal weight was represented by the average weight between beginning and end of the production cycle.

For each farm, from the nADD was obtained the number of Animal Daily Dose administered for each animal in the two years of the study, dividing the nADD by the total number of animal at risk of been treated in each farm:

$$b)\ nADDa = \frac{nADD}{total\ number\ of\ animal\ ar\ risk}$$

Considering that the included farms did not practice the "all-in all-out" system, the total number of animal at risk of treatment included the number of animals present in each farm on the 1st January 2014 and all the animals entering each farm across 2014 and 2015.

The antimicrobial usage records were also processed in order to obtain the Used Daily Dose (UDD) for each drug, representing the dose actually administered to the animals. The UDD of each drug was calculated with the following equation:

$$c)\ \ UDD = \frac{total\ amount\ of\ drug\ administered\ (mg)}{average\ animal\ weight\ (kg)\ x\ number\ of\ applications}$$

The number of applications represented all the treatments applied on animals and was obtained by multiplying the number of animals treated by treatment days (Eq "d").

$$d)\ \ number\ of\ applications = n°\ animals\ treated\ x\ n°\ days\ of\ treatment$$

The UDD/recommended dose (24 h) ratio was calculated to assess the compliance with dosing. A ratio between 0.8 and 1.2 was considered as appropriate. A ratio lower than 0.8 or higher than 1.2 was considered as under- or over-dosing, respectively (Timmerman et al., 2006).

Statistical analysis

Statistical analysis was performed using a freeware statistical software package R v.3.4.1. Categorical variables were reported as frequency, percentage or both, while numerical variables were reported as median (min, max). According to a previous study, three animal groups were formed, basing on the maximal weight difference between average animals weights at arrival (Lava et al., 2016): < 50 kg, 50-100 kg, > 100 kg. The number of Animal Daily Dose for animal was reported as mean ± standard deviation (SD) (median, max), while data about UDD/recommended dose ratio were reported as mean ± standard deviation (SD), min and max and as mean ± standard deviation (SD) of percentage of under, normal or over dosed treatment performed in each farm. Spearman correlation coefficient was used to determine the correlation between the nADD, both total and

divided on type of treatment (individual and group) and numeric variables reported in table 12. The correlation between individual and group treatment was also evaluated. The Wilcoxon rank sum test was used to evaluate difference in nADD, based on categorical variables reported in table 13. For variables including more than two categories, pair-wise comparisons were made using the Wilcoxon rank sum test and the *P* value were corrected with Bonferroni method. When the farm distribution within the different categories was too unbalanced (e.g. “Parasiticides at arrival”: 23 Yes *vs* 3 No), the test was not performed. The variables that significantly influenced the nADD were then tested against each other, in order to evaluate possible associations. *P* was set at 0.05.

RESULTS

Among the 30 farms that agreed to participate at the study, four provided incomplete information about the antimicrobial prescription and were therefore excluded. The final analysis was thus performed on 26 farms, attended by five different veterinary practitioners.

Animals and farm data

Detailed results of the questionnaire are reported in table 12 and 13.

The total number of animals at risk during the two years of the study (2014 and 2015) was 64,719, with a median of animals per farm of 1,771 (301, 8,399). Sixteen of the farms (61.5%) imported only male subjects, one imported only female and nine farms imported both, with a percent of females that ranged from 10% to 70%. Mortality data were available for 23 farms out of 26. The medians of average weight at the end of the cycles was 665 kg (475, 750). All farms had vaccination protocol against virus, while 88.5% (23/26) vaccinated also against bacteria. The median of days between arrival and vaccination was 1 (0, 7). All the farms had a planned quarantine period, during which 7 (26.9%) farms provided to the animals a specific food ration for the adjustment period, while the others 19 (73.1%) farms provided the normal ration planned for the fattening period, but in lower quantity and with hay provided *ad libitum*. In all the farms the animals were checked at least twice a day and in in five (19.2%) these checks were performed by farmers entering the pens. Twenty-three farms had planned veterinary visits. Twenty-three (88.5%) farmers practiced the depopulation period in quarantine locals between batches. All farms had separating fence allowing nose-to-nose contact, and in all farms, there was a straw bedding system.

Table 12. Median, min and max values of detailed results related to animals and farms or 26 beef calves fattening operations located in northwestern Italy. They were reported as median, min and max. [a]Calculated including the 2 farms which had the scraper in quarantine locals. [b,c] Calculated only for the farms without scraper in fattening (n= 14) and infirmary (n= 20) locals. [d,e,f] Calculated only for farms that practiced disinfection in quarantine (n=24), fattening (n=21) and infirmary (n=24) locals.

Parameter	Median	Min	Max
Average number of animals/year	856	151	4,200
Mortality (%)	0.9	0.14	4.94
Average weight at the beginning of production cycle (kg)	346	195	475
Average weight at the end of the production cycle (kg)	665	475	750
Frequency of veterinary visits (days)	7	1	15
Space allowance in quarantine locals (m^2/animal)	4.6	3.4	37.4
Space allowance in fattening locals (m^2/animal)	4.7	3.4	6.8
Space allowance in infirmary locals (m^2/animal)	9.2	2.9	30
Number of animals/pen in quarantine locals	14	6	60
Number of animals/pen in fattening locals	9	5	20
Number of animals/pen in infirmary locals	3	1	9
Frequency of straw adding (days)	1.9	1	17
Amount of straw added per animal per day (kg)	3.1	1	5.7
Cleaning frequency in quarantine locals (days)[a]	20	10.5	91
Cleaning frequency in fattening locals (days)[b]	20.5	12.5	53
Cleaning frequency in infirmary locals (days)[b]	20.5	10	60
Disinfection frequency in quarantine locals (days)[d]	20.5	15	365
Disinfection frequency in fattening locals (days)[e]	53	12.5	365
Disinfection frequency in infirmary locals (days)[f]	25	1	365
Duration of depopulation period in quarantine locals (days)	7	0	30

3576 *Table 13: Characteristics of farms and animals of 26 beef calves fattening operations located in*
3577 *northwestern Italy, reported as frequency.*

Parameter	Category	N° farms	Percent
Purchasing at least the 10% of females	Yes	10	38.5%
	No	16	61.5%
Maximal weight difference between average animals' weight at arrival (kg)	<50	7	26.9%
	50-100	13	50%
	>100	6	23.1%
Pre-arrival health information	Yes	1	3.8%
	No	25	96.2%
Thorough physical examination at arrival	Yes	8	30.8%
	No	18	69.2%
Vaccination against bacteria	Yes	23	88.5%
	No	3	11.5%
Interval longer than 1 day between arrival and vaccination	Yes	10	38.5%
	No	16	61.5%
Parasiticides at arrival	Yes	23	88.5%
	No	3	11.5%
Regularly prophylactic/metaphylactic treatment at arrival	Yes	15	57.7%
	No	11	42.3%
Specific diet provided for animals in the quarantine period	Yes	7	26.9%
	No	19	73.1%
Presence of a restraint cage	Yes	9	34.6%
	No	17	65.4%
Animals handling corridor	Yes	14	53.8%
	No	12	46.2%
Fattening group divided on the base of origins or arrival	Yes	5	19.2%
	No	21	80.8%
Fattening group divided on the base of weight at arrival	Yes	24	92.3%
	No	2	7.7%
Daily animals' checks entering the pen	Yes	5	19.2%
	No	21	80.8%
All BRD cases are examined by the veterinary practitioner before treatment	Yes	9	34.6%
	No	17	65.4%
The animals are moved in the infirmary locals at the first onset clinical signs	Yes	5	19.2%
	No	21	80.8%
Mechanical ventilation in closed locals	Yes	23	88.5%
	No	3	11.5%
Open quarantine locals	Yes	17	65.4%
	No	9	34.6%
Isolated quarantine locals	Yes	23	88.5%
	No	3	11.5%
Paddock in quarantine locals	Yes	6	23.1%
	No	20	76.9%
Open fattening locals	Yes	3	11.5%
	No	23	88.5%
Paddock in fattening locals	Yes	6	23.1%
	No	20	76.9%

Open infirmary locals	Yes	11	42.3%
	No	15	57.7%
Infirmary locals isolated from the rest of the structure	Yes	8	30.8%
	No	18	69.2%
Paddock in infirmary locals	Yes	1	3.8%
	No	25	96.2%
Scraper in at least one local	Yes	13	50%
	No	13	50%
Scraper in quarantine locals	Yes	2	7.7%
	No	24	92.3%
Quarantine locals disinfection	Yes	24	92.3%
	No	2	7.7%
Scraper in fattening locals	Yes	12	46.2%
	No	14	53.8%
Bedding removing in fattening locals	Scraper	6	23.1%
	Scraper and manually	6	23.1%
	Manually	14	53.8%
Fattening locals disinfection	Yes	21	80.8%
	No	5	19.2%
Scraper in infirmary locals	Yes	6	23.1%
	No	20	76.9%
Bedding removing in infirmary locals	Scraper	3	11.5%
	Scraper and manually	3	11.5%
	Manually	20	77%
Infirmary locals disinfection	Yes	24	92.3%
	No	2	7.7%
Depopulation period in fattening locals	Yes	2	7.7%
	No	24	92.3%
Depopulation period in infirmary locals	Yes	11	42.3%
	No	15	57.7%

Antimicrobial consumption data

Overall, 821.7 kg of antimicrobials were used in the 26 farms during 2014 and 2015; 57.2% of them were orally administered and were mostly composed by tetracyclines (91.4%). Even if the amount (kg) of parenterally administered antimicrobials was lower (42.8%) than the orally administered, there was more variability in the number of substance group used. The most used antimicrobials in parenteral administration were fenicoles (38.7%) and macrolides (23.8%) (table 14).

Table 14. Consumption of active substances by specified substance groups and administration route (oral and parenteral) from 26 fattening operations located in northwestern Italy.

Substance group	Parenteral			Oral			Total		
	N° farms	kg	%	N°	kg	%	N° farms	kg	%
Aminoglycosides	22	20.1	5.7	0	0	0	22	20.1	2.4
Penicillines	22	22.9	6.5	0	0	0	22	22.9	2.8
Cephalosporines	12	3.1	0.9	0	0	0	12	3.1	0.4
Fenicoles	24	136.1	38.7	0	0	0	24	136.1	16.5
Fluoroquinolones	25	23.48	6.7	1	0.04	0.01	25	23.52	2.9
Lincosamides	18	8.4	2.4	0	0	0	18	8.4	1
Macrolides	26	83.7	23.8	0	0	0	26	83.7	10.2
Polymyxines	3	0.15	0.1	0	0	0	3	0.15	0.1
Sulfonamides	15	14.8	4.2	0	0	0	15	14.8	1.8
Sulfonamide and Trimethoprim	15	18.4	5.2	5	40.3	8.6	17	58.7	7.1
Tetracyclines	20	20.4	5.8	17	429.7	91.39	21	450.1	54.8
All	26	351.6	42.8	17	470.1	57.2	-	821.7	100

Overall, the mean (± SD, median, max) of daily dose animal (nADD) used during the study period in each farm was 3 (± 2.1, 2.6, 8.3). Mean (± SD, median, max) of nADD for group treatment was 1.7 (± 1.9, 1.2, 6.8) and antimicrobial treatments were administered orally in the 70.4% of the cases and parenterally in the 29.6%. Oral formulations were composed primarily by doxycycline (97%). Parental formulations were composed mainly of long-acting macrolides, such as tulathromycin (41.5%) and tildipirosin (26.8%), and formulations containing florfenicol (6.8%), alone or in association with flunixin meglumide. Mean (± SD, median, max) of nADD for individual treatment was 1.3 (± 0.7, 1.4, 3), and they were principally administered parenterally (98.1%). The most used molecules included florfenicol (19.9%), marbofloxacin (19.5%) and tylosin (12.4%). Further details about the molecules used in the farms are reported in table 15.

Table 15. Distribution, mean ± standard deviation (SD), median and maximum value of number of daily dose animal (nADD) administered during 2014 and 2015 in 26 fattening operations located in northwestern Italy. They were reported for group and individual treatment and based on the administration way (oral, parenteral). The number of farms using the drug is also reported ("N° farms"). The Long-acting (LA) factors and the final ADD (mg/kg) value used for the analysis were reported.

Parameters used for the analysis				**Results**				
					nADD			
Molecule	**ATC-vet**	**LA factor**	**ADD**	**N° farms**	**%**	**Mean ± sd**	**Median**	**Max**
GROUP TREATMENT					57.4			
Oral formulation					70.4			
Docycycline	QJ01AA02	1	10	17	97	1.17 ± 1.7	0.3	5.6
Sulfadiazine/trimethoprim	QJ01EW10	1	24	5	3	0.04 ± 0.1	0	0.4
Parenteral formulation					29.6			
Aminosidin	QJ01GB92	1	10.5	1	0.8	0.1	0	0.1
Amoxicillin	QJ01CA04	1 - 2	7.25	1	0.08	0.01	0	0.01
Enrofloxacin	QJ01MA90	1 -2	4.4	3	3.2	0.02 ± 0.07	0	0.4
Florfenicol-Florfenicol + flunixin	QJ01BA90/ QI01BA99	2 - 4	12.5	13	6.8	0.04 ± 0.08	0.005	0.4
Lincomycin/spectinomycin	QJ01FF52	1	15	4	0.2	0.001 ± 0.005	0	0.02
Marbofloxacin	QJ01MA93	1 - 4	2	8	6.2	0.03 ± 0.1	0	0.5
Oxytetracycline	QJ01AA06	1 - 2	6.5	7	4	0.02 ± 0.09	0	0.4
Procaine benzylpenicillin	QJ01CE09	1	12	1	0.04	0.006	0	0.006

Spiramycin	QJ01FA02	2	15.6	5	1.7	0.008 ± 0.02	0	0.08
Sulfadimidine/Trimethoprim	QJ01EW03	1	15.5	1	0.2	0.03	0	0.03
Sulfamonomethoxine	QJ01EQ18	1	40	1	0.06	0.008	0	0.008
Thiamphenicol	QJ01BA02	1	37.5	1	0.03	0.004	0	0.004
Tildipirosin	QJ01FA96	5	0.8	13	26.8	0.14 ± 0.4	0.006	1.6
Tilmicosin	QJ01FA91	1 - 2	6	6	5.8	0.03 ± 0.1	0	0.5
Tylosin	QJ01FA90	1	7	4	2.5	0.01 ± 0.04	0	0.2
Tulathromycin	QJ01FA94	5	0.5	12	41.5	0.2 ± 0.4	0	1.8
INDIVIDUAL TREATMENT					42.6			
Oral formulation					1.9			
Docyciclyne	QJ01AA02	1	10	6	98.8	0.02 ± 0.09	0	0.4
Enrofloxacin	QJ01MA90	1	3.75	1	1.2	0.008	0	0.008
Parenteral formulation					98.1			
Aminosidin	QJ01GB92	1	10.5	2	0.2	0.003 ± 0.01	0	0.05
Amoxicillin	QJ01CA04	1 - 2	7.25	13	3.9	0.05 ± 0.07	0.02	0.3
Ampicillin	QJ01CA01	1	7.5	6	1.4	0.02 ± 0.05	0	0.2
Ampicillin/colistin sulphate	QJ01RV01	1	11.3	3	0.3	0.003 ± 0.01	0	0.04
Ampicillin/dicloxacillin	QJ01CR50	1	10.7	7	0.9	0.01 ± 0.03	0	0.2
Procaine benzylpenicillin/dihydrostreptomycin	QJ01RA01	1	43	3	0.1	0.002 ± 0.006	0	0.03
Cefquinome	QJ01DE90	1	1	4	1.2	0.02 ± 0.06	0	0.3
Ceftiofur	QJ01DD90	1 – 6	1	9	6.3	0.08 ± 0.3	0	1.2
Danofloxacin	QJ01MA92	2	3	2	0.07	0.0009 ± 0.004	0	0.02

Enrofloxacin	QJ01MA90	1 - 2	4.4	11	6.4	0.08 ± 0.2	0	0.8
Erythromycin/sulfamonomethoxine	QJ01RA91	1	25	1	0.05	0.02	0	0.02
Florfenicol-Florfenicol + flunixin	QJ01BA90/QI01BA99	2 - 4	12.5	22	19.9	0.26 ± 0.2	0.3	0.6
Gamithromycin	QJ01FA95	5	1.2	1	0.01	0.003	0	0.003
Kanamycin	QJ01GB04	0.5	13.5	5	0.5	0.008 ± 0.03	0	0,2
Lincomycin/spectinomycin	QJ01FF52	1	15	18	2.9	0.04 ± 0,05	0.02	0.1
Marbofloxacin	QJ01MA93	1 - 4	2	18	19.5	0.2 ± 0.3	0.2	1
Oxytetracycline	QJ01AA06	1 - 2	6.5	20	6.3	0.08 ± 0.08	0.08	0.3
Procaine benzylpenicillin	QJ01CE09	1	12	1	0.06	0.02	0	0.02
Spiramycin	QJ01FA02	2	15.6	16	6.6	0.08 ± 0.1	0.05	0.4
Sulfadiazine/trimethoprim	QJ01EW10	1	24	1	0.09	0.04	0	0.04
Sulfadimethoxine	QJ01EQ09	1	31	4	0.3	0.003 ± 0.01	0	0.05
Sulfadimidine/sulfadimethoxine/trimethoprim	QJ01EW03	1	15.5	1	0.02	0.007	0	0.007
Sulfadimidine/trimethoprim	QJ01EW03	1	15.5	15	2.3	0.03 ± 0.04	0.01	0.1
Sulfametopyrazine	QJ01EQ19	1	36	1	0.3	0.08	0	0.08
Sulfamonomethoxine	QJ01EQ18	1	40	10	0.6	0.008 ± 0.02	0	0.06
Thiamphenicol	QJ01BA02	1	37.5	8	0.5	0.006 ± 0.02	0	0.06
Tildipirosin	QJ01FA96	5	0.8	12	5.4	0.07 ± 0.09	0	0.3
Tilmicosin	QJ01FA91	1 - 2	6	15	0.8	0.01 ± 0.02	0.005	0.06
Tylosin	QJ01FA90	1	7	23	12.4	0.15 ± 0.2	0.1	0.8
Tulathromycin	QJ01FA94	5	0.5	6	0.7	0.009 ± 0.03	0	0.1

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Considering the whole treatment, the mean of the UDD/recommended dose ratio was 0.9 (± 0.26; 0.56, 1.62). The average UDD/recommended dose ratio for group treatment was 0.77 (± 0.29; 0.24, 1.23). On average, 23.5% (± 35.9%) of group treatment administered orally were under dosed, 41.2% (± 44.1%) were administered at the correct dosage and 35.3% (± 49.3%) were over dosed. Regarding the parenterally administered group treatment, 75.5% (± 34.3%) were under dosed, 17.9% (± 30.4%) were administered at the correct dosage and 6.5% (± 14.6%) were over dosed. The mean of UDD/recommended dose ratio for individual treatment was 0.98 (± 0.26; 0.58, 1.62). The individual orally administered treatment was under dosed in 14.3% (± 37.8%) of the cases, administered at correct dosage in 28.57% (± 48.8%) and over dosed in 57.1% (± 53.5%). 48% (± 21.9%) of the parenterally administered individual treatment was under dosed, while 24.7% (± 13.3%) was correctly administered and 27.4% (± 17%) over dosed. Further details regarding the UDD/recommended dose ratio were reported in table 16.

3621 *Table 16. Recommended dose (mg/kg) approved by pharmaceutical companies and dosing ratio of individual and group antimicrobial treatments,*
3622 *based on administration route, in 26 Piedmontese fattening operations. More than one recommended doses were reported when more than one*
3623 *commercial formulations of the same molecules were present (e.g. long-acting and normal preparations). RD = Recommended dose.*

3624

Molecule	ATC-vet	RD	Mean ± sd (min, max)		N° farms		
			UDD	UDD/RD	< 0.8	0.8-1.2	>1.2
GROUP TREATMENT							
Oral formulation							
Docycycline	QJ01AA02	10	12.5 ± 5.7 (5.8, 29.3)	1.2 ± 0.6 (0.6, 2.9)	2	9	6
Sulfadiazine/Trimethoprim	QJ01EW10	24	12.8 ± 3.6 (7.7, 17.2)	0.5 ± 0.2 (0.3, 0.7)	5	-	-
Parenteral formulation							
Aminosidin	QJ01GB92	10.5	2	0.2	1	-	-
Amoxicillin	QJ01CA04	15	0.5	0.04	1	-	-
Enrofloxacin	QJ01MA90	5 – 7.5	1.5 ± 0.5 (1.2, 2)	0.3 ± 0.1 (0.1, 0.4)	3	-	-
Florfenicol/Florfenicol + flunixin	QJ01BA90/QI01BA99	20 - 40	11.5 ± 5.2 (3.1, 19.3)	0.5 ± 0.2 (0.2, 1)	12	1	-
Lincomycin/Spectinomycin	QJ01FF52	15	6.1 ± 2.2 (3.6, 9)	0.4 ± 0.2 (0.2, 0.6)	4	-	-
Marbofloxacin	QJ01MA93	2 - 8	4.5 ± 3.4 (1, 11)	1.2 ± 1.3 (0.1, 3.8)	4	1	3
Oxytetracycline	QJ01AA06	4.7 – 7 - 20	3.1 ± 2.9 (0.3, 7.1)	0.4 ± 0.5 (0.06, 1.4)	6	-	1
Procaine benzylpenicillin	QJ01CE09	12	12	1	-	1	-
Spiramycin	QJ01FA02	31.25	7 ± 4.9 (1.7, 13.7)	0.2 ± 0.2 (0.05, 0.4)	5	-	-
Sulfamidine/Trimethoprim	QJ01EW03	15	11	0.7	1	-	-
Sulfamonomethoxine	QJ01EQ18	40	2.5	0.06	1	-	-
Thiamphenicol	QJ01BA02	37.5	12.5	0.3	1	-	-
Tildipirosin	QJ01FA96	4	2.8 ± 1 (1, 4.3)	0.7 ± 0.3 (0.3, 1.1)	8	5	-
Tilmicosin	QJ01FA91	10	6.6 ± 2 (4.5, 10.4)	0.7 ± 0.2 (0.5, 1)	5	1	-
Tylosin	QJ01FA90	7	7.7 ± 6.4 (2, 14.5)	1.1 ± 0.9 (0.3, 2.1)	2	-	2

Tulathromycin	QJ01FA94	2.5	1.8 ± 0.4 (1.2, 2.5)	0.7 ± 0.2 (0.5, 1)	8	4	-
INDIVIDUAL TREATMENT							
Oral formulation							
Docyciclyne	QJ01AA02	10	22.1 ± 15.3 (9.3, 47)	2.2 ± 1.5 (0.9, 4.7)	-	-	4
Enrofloxacin	QJ01MA90	3.75	1.9	0.5	1	-	-
Parenteral formulation							
Aminosidin	QJ01GB92	10.5	8.6 ± 2.3 (7, 10.2)	0.8 ± 0.2 (0.7, 1)	1	1	-
Amoxicillin	QJ01CA04	7 - 15	9.8 ± 3.3 (6.6, 18.3)	1 ± 0.6 (0.4, 2.6)	5	5	3
Ampicillin	QJ01CA01	7.5	13.1 ± 5.5 (4.9, 21.4)	1.7 ± 0.7 (0.7, 2.9)	1	-	5
Ampicillin/colistin sulphate	QJ01RV01	11.2	6.5 ± 1.5 (4.8, 7.3)	0.6 ± 0.1 (0.4, 0.7)	3	-	-
Ampicillin/dicloxacillin	QJ01CR50	10.7	10.5 ± 3.1 (6.9, 15.6)	1 ± 0.3 (0.6, 1.5)	2	3	2
Procaine benzylpenicillin/Dihydrostreptomycin	QJ01RA01	19.5 - 40	20 ± 2 (18.3, 22.2)	0.7 ± 0.4 (0.5, 1.3)	2	1	-
Cefquinome	QJ01DE90	1	1 ± 0.3 (0.5, 1.3)	1 ± 0.3 (0.5, 1.3)	1	2	2
Ceftiofur	QJ01DD90	1 – 6.6	3.5 ± 2.6 (0.9, 7.8)	1.7 ± 2.1 (0.5, 7.2)	3	4	2
Danofloxacin	QJ01MA92	6	6.3 ± 0.9 (5.7, 6.9)	1.1 ± 0.1 (1, 1.2)	-	2	-
Enrofloxacin	QJ01MA90	5 – 7.5	3.1 ± 1 (1.4, 5.2)	0.6 ± 0.2 (0.2, 1)	10	1	-
Erythromycin/Sulfamonomethoxine	QJ01RA91	25	13	0.5	1	-	-
Florfenicol/Florfenicol + flunixin	QJ01BA90/QI01BA99	20 – 30 - 40	15.5 ± 4 (8.1, 22.2)	0.7 ± 0.2 (0.4, 1.1)	16	4	-
Gamithromycin	QJ01FA95	6	3.6	0.6	1	-	-
Kanamycin	QJ01GB04	16.5	6.2 ± 1.9 (4, 8.6)	0.4 ± 0.1 (0.3, 0.5)	2	2	1
Lincomycin/spectinomycin	QJ01FF52	15	9.5 ± 2.6 (5.6, 13.6)	0.6 ± 0.2 (0.4, 1)	14	4	-
Marbofloxacin	QJ01MA93	2 - 8	4.8 ± 2.1 (1.6, 10.2)	1.1 ± 0.9 (0.2, 3.1)	7	5	6
Oxytetracycline	QJ01AA06	4.7 -7 - 20	7.3 ± 2.3 (3.9, 13.5)	1.3 ± 0.5 (0.6, 2.3)	6	4	11
Procaine benzylpenicillin	QJ01CE09	12	12.4	1.1	-	1	-
Spiramycin	QJ01FA02	31.25	11.2 ± 2.1	0.4 ± 0.07 (0.3, 0.5)	16	-	-
Sulfadiazine/trimethoprim	QJ01EW10	20	29.9	1.5	-	-	1

Sulfadimethoxine	QJ01EQ09	31	28.3 ± 18.8 (4.4, 50.3)	0.9 ± 0.6 (0.1, 1.6)	1	2	1
Sulfamidine/ sulfadimethoxine/trimethoprim	QJ01EW03	14.4	11.7	0.8	-	1	-
Sulfadimidine/trimethoprim	QJ01EW03	15 - 16	17.9 ± 3.9 (11.6, 24.3)	1.2 ± 0.3 (0.8, 1.6)	1	5	9
Sulfametopyrazine	QJ01EQ19	36	21.4	0.6	1	-	-
Sulfamonomethoxine	QJ01EQ18	40	33.5 ± 8.6 (23.5, 52.1)	0.8 ± 0.2 (0.6, 1.3)	6	3	1
Thiamphenicol	QJ01BA02	37.5	14.2 ± 3 (9.3, 18.3)	0.4 ± 0.08 (0.3, 0.5)	8	-	-
Tildipirosin	QJ01FA96	4	4.1 ± 0.9 (2.9, 5.8)	1 ± 0.2 (0.7, 1.5)	1	9	2
Tilmicosin	QJ01FA91	7 - 10	7.2 ± 3.6 (2.9, 15.2)	0.7 ± 0.4 (0.3, 1.5)	10	3	2
Tylosin	QJ01FA90	7	11.6 ± 2.4 (7.4, 15.9)	1.7 ± 0.4 (1.1, 2.3)	-	9	14
Tulathromycin	QJ01FA94	2.5	2.2 ± 0.4 (1.5, 2.7)	0.9 ± 0.2 (0.6, 1.1)	1	5	-

Associations between antimicrobial consumption and farms characteristics

No associations were found between mortality and number of daily dose (nADD) used in surveyed years, both considered as a whole and grouped on type of treatment (individual or group) ($P > 0.05$). No association was found between group and individual nADD ($P > 0.05$). Statistical differences in number of ADD were found on the application of regular prophylactic/metaphylactic treatment at arrival for each group and total treatment, but not for individual one. In fact, farms that regularly practiced prophylactic/metaphylactic treatment had higher nADD ($P < 0.05$) for group (2.6 ± 2; 2, 6.8) and total treatment (3.8 ± 2.3; 3.3, 8.3), when compared to farms which did not practiced prophylactic/metaphylactic treatment at arrival (group: 0.5 ± 0.6; 0.1, 1.8; total: 1.8 ± 1.1; 1.5, 4.4). Moreover, a negative correlation was found between the nADD and the average batch weight at arrival for total ($P < 0.05$, r = -0.44) and group treatment ($P < 0.05$, r = -0.51). Furthermore, a negative correlation was found between the nADD of group treatment and the kg of straw added per day per animal ($P < 0.05$, r = -0.43). Comparing the variables that were significantly correlated with the nADD used, an association within regularly prophylactic/metaphylactic treatment was found. The median average batch weight at arrival was lower (310 kg; 195, 450) in farms that used regularly prophylactic/metaphylactic treatment, when compared to the other farms (400 kg; 280, 475). No other differences were found based on the analyzed categories. Nevertheless, a tendency to significance was found for nADD for individual treatment, based on performing thorough physical examination at arrival ($P = 0.054$) and on moving animals in the infirmary locals at the first onset of clinical signs ($P = 0.067$). In fact, a lower nADD value was found in both farms that performed a thorough physical examination at arrival (0.9 ± 0.4; 0.8, 1.6) and ones that moved the animals at the onset of clinical signs (0.8 ± 0.6; 0.8, 1.5), compared with the others (1.4 ± 0.7; 1.5, 3 and 1.4 ± 0.7; 1.5, 3, respectively). Moreover, the cleaning frequency in fattening locals without scraper tended to be positive correlated with the individual treatment nADD ($P = 0.05$, r = 0.5). All the nADD for total, individual and group treatment for every parameter and every category were reported in Appendix5, tableS6.

DISCUSSION

To the author's knowledge, there are few studies of antimicrobial use, expressed in nADD, in beef cattle fattening operations (Carson et al., 2008; Caucci et al., 2018). The nADD calculation method allowed to determine the amount of antimicrobial use, without the influence of animal weight or difference in potency between active compounds (Jensen et al., 2004). However, this calculation requires the weight of the animals at the time of antimicrobial administration (MARAN, 2011) and as the exact animal weight estimation at treatment time is hard to achieve, average weight for animal categories have been proposed in literature (Jensen et al., 2004; MARAN, 2011, 2012). For beef cattle, three different weights have been suggested: 300 Kg, 500 Kg, 600 Kg (Jensen et al., 2004;

MARAN, 2011, 2012). In the author's opinion, farms included in the present study have overly broad weight intervals and none of the values proposed by literature fitted well with the whole farms. Consequently, a mean between the beginning and the end of the fattening period was chosen. A similar choice has been proposed in other studies (MARAN, 2017; Caucci et al., 2018). The average nADD used per farm during the whole study period was 3. The values of nADD reported for poultry, pigs and veal calves were usually higher than the one reported for adult cattle. In a German study with data collection in 2006 and 2007, the higher nADD reported were for piglets (60.86), fattening pigs (28.6) and calves (8.33), while dairy and beef cattle recorded lower nADD: 2.75 and 0.08, respectively (Merle et al., 2012). In Netherlands, as in Germany, the categories with the highest amount of antimicrobials, expressed in nADD, were veal calves (20.88 in 2016) and pigs (8.87 in 2016), in addition to broilers (10.19 in 2016), turkeys (26.42 in 2016) and rabbits (40.93 in 2016) (MARAN, 2017). In beef cattle the reported nADD were lower, compared to the present study (1.07 in 2016, 1.15 in 2014 and 1 in 2015) (MARAN, 2017). However, both German and Dutch studies did not divide the beef cattle in different production category (cow-calf or fattening units). Consequently, the presence of other production categories (e.g. as cow-calf operations) may have mitigated the nADD found for beef cattle. In Wisconsin, USA, a 2007 study performed on 20 conventional farms, reported the use of 5.43 nADD/cow for year (Pol and Ruegg, 2007). Similar results were obtained in Netherlands, where a 7 year study, performed in 94 dairy farms, reported an average nADD of 5.86 (Kuipers et al., 2016). However, more recent data reported lower nADD for dairy herds in Netherlands (3 in 2016) (MARAN, 2017).

Overall, the group treatment included more than a half of total nADD (57.4%) and most of them were performed orally (70.4%). However, considering the whole treatment, the ones administered orally represented 41.2%. This percentage is low, compared to the results found in literature. In veal calves, both the percentage of group and oral treatments were higher. Lava et al., reported a 84.6% of group treatments, 94.8% of which administered orally (Lava et al., 2016), similar as Jarrige et al., who reported a 95.8% of group treatments, 96.8% of which administered orally (Jarrige et al., 2017). In Belgium, most of the treatment, both antimicrobials and anti-inflammatory, performed on veal calves were group treatment (97.7%), and all the antimicrobials group treatment (82%) were administered orally (Pardon et al., 2012b). Merle et al., recorded higher oral antimicrobial usage not only in calves, but also in beef cattle (Merle et al., 2014). Contrarily, in Denmark, the percentage of oral treatments in veal calves and young bulls was low (14.6%) (Fertner et al., 2016). Moreover, in Ontario feedlots (Canada), the amount of kg orally administered was higher than the parenteral one (Carson et al., 2008).

The main active compound in oral treatment was doxycycline. Tetracyclines are the most important orally administered antimicrobials in Belgian veal calves and in Danish veal calves and young bulls (Pardon et al., 2012b; Catry et al., 2016; Fertner et al., 2016). Oral tetracyclines are registered for BRD control, even if, doxycycline is registered for oral use in calves with immature rumen function,

where its availability and pharmacokinetics have been evaluated (Meijer et al., 1993). To the author's knowledge, no studies regarding the adoption of this molecules in weaned calves were carried out, since the presence of a completely functional rumen may alter the drug pharmacokinetics, with a possible dilution effect by ruminal fluid.

In group treatment with parenteral administration, the most used molecules were tulathromycin and tildipirosin, two long-acting macrolides registered for BRD individual and metaphylactic treatment (Draxxin® and Zuprevo®, respectively). They have been largely used in BRD prevention and treatment, thanks to their pharmacokinetics which include an extended distribution in pulmonary tissue and a slow elimination (Godinho et al., 2005; Menge et al., 2012). Tulathromycin was largely used for group treatment also in Swiss (Lava et al., 2016). Moreover, macrolides were highly used in North-Eastern Italian fattening operations, but it is not reported what percentage they were administered for group or individual treatment (Caucci et al., 2018).

Oral formulations for individual treatment were used in very few cases, as it has been reported for Swiss veal calves (Lava et al., 2016). Individual treatment, in fact, were mainly administered parenterally, the three most employed substances being florfenicol, marbofloxacin and tylosin. In Italy, florfenicol is registered for treatment of respiratory disease in cattle, marbofloxacin for respiratory disease and mastitis, while tylosin has multiple indication, including respiratory disease, mastitis, metritis and foot rot (EMA, 1996a; b, 1997). Florfenicol was one of the most used antimicrobial used for individual parenteral treatment also in veal calves in Belgium (Pardon et al., 2012b). Moreover, it was largely used in northeastern Italian fattening operations as well, and in Ontario feedlot (Carson et al., 2008; Caucci et al., 2018). Fluoroquinolone were among the most frequently used antimicrobials in Swiss veal calves, while tylosin was largely used in group treatment in both Swiss and Belgian veal calves (Pardon et al., 2012b; Lava et al., 2016).

Mostly of parenteral treatment, both group or individual, were administered under-dosed. Even if the weight estimation may not have been precise, possibly influencing the dosing evaluation, several values of UDD/recommended dose ratio found in the present study were too low, even considering a lower weight. A similar tendency in under-dosing, variable on the base of considered molecules and treatment type, was observed in other studies focused on monitoring of antimicrobial usage in cattle and pigs (Timmerman et al., 2006; Pardon et al., 2012b; Merle et al., 2014; Caucci et al., 2018). This could be correlated with an underestimation of body weight at the moment of treatment (Pardon et al., 2012b). Nevertheless, antimicrobial under-dosing has proved to predispose pathogen antimicrobial resistance, yielding in great danger for both animal and human health (Roberts et al., 2008; Catry et al., 2016). Furthermore, group antimicrobial administration, mostly orally, in feedlot and veal calves has been correlated with increased bacterial resistance in feces (Checkley et al., 2010; Duse et al., 2015; Catry et al., 2016). Moreover, selected molecules, such as oxytetracyclines, seemed to be more involved in antimicrobial resistance and co-resistance (Lubbers and Hanzlicek, 2013). Many antibiotic resistance in BRD pathogens, for instance, was found to tetracyclines and

macrolides, which have been largely used in the present study, and the abuse of this antibiotic class has been correlated with antibiotic resistance in human pathogens (Portis et al., 2012; Lubbers and Hanzlicek, 2013; ECDC/EFSA/EMA, 2017). Also fluoroquinolones use in food-producing animals was associated with resistance in human pathogens (ECDC/EFSA/EMA, 2017). Fluoroquinolones, as well as macrolides, have both been largely used in the present study and are considered as *highest priority critically important antimicrobials*, according to the WHO classification (World Health Organization, 2016). Consequently, the proposed guidelines by European Union are aimed at reducing the use of these antimicrobial classes in animals productions (EFSA/ECDC/EMA, 2017).

In the present study, comparing nADD usage with several management and structural factors, few associatinos were found. The fact that regular application of prophylactic/metaphylactic treatment at arrival influenced the nADD for group treatment is easy to understand, but it is also worth noting that it influenced the total nADD. Moreover, both total and group nADD were correlated with average batch weight at arrival. Considering that prophylactic/metaphylactic treatment resulted to be more applied on lighter calves, this correlation could be the consequence of a management choice to administer prophylactic/metaphylactic treatment in younger animals, considered at higher risk (Taylor et al., 2010).

At the same time, individual nADD did not change on the base of prophylactic/metaphylactic treatment at arrival, nor they were correlated with group nADD. Moreover, mortality was not correlated with total, group or individual nADD usage. Following the results of the present study, the application of prophylactic/metaphylactic group treatment at arrival did not seem to reduce the further individual antimicrobial treatment or influenced herd mortality. Group treatments have been applied mostly in animals' categories with higher risk of developing BRD, which could explain why no differences were found.

A reduced use of straw bedding per animal was correlated with higher use of antimicrobial for group treatment. The amount of added straw per animal per day influenced humidity and hygiene of pens, and it contributed in reducing the cold stress during winter season (Canali et al., 2001; Mader, 2003). Moreover, a higher amount of straw was proved to reduce emission of ammonia (Gilhespy et al., 2009). The latter has been reported to impair local respiratory defense, predisposing to rhinitis in mice and swine, and it has been suggested that it may exert similar effects on cattle (Hamilton et al., 1996; Caswell, 2014). Consequently, its scarcity may contribute in predisposing the animals to BRD development. But this relation could probably be explained in a converse way, i.e. farmers which had used less straw per animal per day could have experienced a higher BRD incidence and consequently predisposed more group treatment for BRD control.

The difference in individual treatment nADD based on a thorough examination at arrival and moving the animals at the onset of clinical signs were not significant but tended to be. However, is not unlikely to postulate that these practices could lead to a reduced amount of individual treatment. Performing

a thorough physical examination at arrival may allow to early identification of affected animals, leading to higher treatment efficacy of and chronic cases reduction (McGuirk and Peek, 2014; Lhermie et al., 2016). Moreover, moving animals to infirmary locals at the onset of clinical signs, i.e. without waiting for the treatment response, reduced the contact between healthy and diseased animals, eventually decreasing the exposure to pathogens in susceptible calves (Edwards, 2010).

Furthermore, a tendency to significance was also found for the positive correlation between the cleaning frequency in fattening local and the individual treatment nADD. This means that farms that had left the bedding for a longer period of time, administered more antimicrobial in individual treatment. Even if this difference tended to significance, it is not unlikely to believe that cleaner farms were correlated with lesser antimicrobial use. In fact, the permanence of the litter for a longer period may increase humidity and ammonia, as well as the bacterial amount in environment, which contributes to pathogen exposure (Callan and Garry, 2002).

The fact that few correlations between management and structure and BRD were found may lie in the fact that, albeit with little difference, the farms included in the present study were sufficiently homogeneous. They were supervised by practitioners that were in collaboration and who probably followed a common line in recommendations given to the farmers.

The use of only treatment records to monitor antimicrobial use could be a limit, considering that this approach has been generally accepted to underestimate antimicrobial use, whereas some records could be missing. As already described, Italian law imposes that the treatment records be signed by veterinary practitioners working in the farms and be checked once a year by veterinarians working in public sector, together with prescriptions and drug stock records. Through prescriptions and antimicrobial stock records, in fact, it was possible to perform a double checking with treatment records. Moreover, the fact that many antimicrobial drugs resulted to be administered underdosed strengthens the assumption that the use of treatment records represented a minor limitation in the present study.

In conclusion, in the light of the results of the present study, beef calves fattening operations seemed less involved than veal calves, pigs and broilers in antimicrobial usage in food-production animals. The main active compounds used are all registered for BRD treatment. Moreover, critical antimicrobials for human medicine were largely used. This study, also, highlighted the tendence to underdose antimicrobials, most of all ones administered orally. Further study including more heterogeneous reality are needed in order to identify others possible factors correlated to antimicrobial usage.

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GENERAL CONCLUSIONS

The results of the primary project of this PhD showed that thoracic ultrasonography could be a more accurate method for BRD diagnosis also in beef calves, underlying the importance of including the most cranial areas in the examination and of scanning both side of the thorax. Moreover, the use of new sequencing technology, the Next Generation Sequencing (NGS), provided the means to make a more detailed description of the respiratory microbiota. The present study showed the presence of a lower respiratory tract microbiota, distinct from the upper one. This confirms that the bovine lung is not sterile, in agreement with the results obtained in human medicine. Upper and lower respiratory tract microbiotas shared bacterial groups, even if differently represented. This indicates that the lung microbiota could likely be self-sustaining, but its composition is probably influenced by that of the upper respiratory tract. Significant differences, based on the presence of ultrasonographic lung consolidation were found only for certain taxa, and not for alpha and beta diversity. This could be explained by the low number of included animals, and a significant difference in beta and alpha diversity could be achieved by increasing the studied subjects. The outcome of this project, along with similar recent studies, point towards the development of alternative methods for BRD control (e.g. probiotics drugs) and suggest the existence of other actors correlated with BRD development, separate from those proposed so far (e.g. geographical provenience).

The first secondary project, conducted on beef calves imported from France, pointed out transportation and winter season as the most important predisposing factors for BRD treatment in the first 60 days on feedlot. Moreover, it intensified the interest on the evaluation of ROMs concentration in arriving animals, as a suitable biomarker for future BRD treatment. However, very few studies have evaluated ROMs concentration in bovine species, and more are needed in order to fully understand how these metabolites are involved in lung inflammatory status and how they could be used as biomarkers for BRD development.

The following secondary project showed beef calves fattening operations to have a lower antimicrobial use, expressed in nADD, when compared with poultry, pigs, veal calves and dairy calves. It is worth noting, though, that such comparison was drawn by analysing data from foreign breedings, due to the lack of Italian reports. Half of the treatments were group treatments, and they were mostly orally administered. The main used active compunds, for both group and individual treatment, were registered for BRD control and treatment. Moreover, a tendency to under-dosing was recorded, for both group and individual treatment, which had been previously reported as associated to higher antimicrobial resistance development. The group treatments were performed primarily on lighter animals, therefore influencing the final number of nADD. Very few factors were recognized to affect the antimicrobial use, probably given the relative homogeneity of the selected farms, which showed little differencies.

APPENDICES

APPENDIX 1

Table S1. Relative abundance of genera identified in the nasal swab (NS) and the trans-tracheal aspiration (TTA) samples. Data are reported as average relative abundance and standard error of the mean (SEM). Genera identified only in the NS (n = 11) or in the TTA (n = 17) samples are shown in bold or underlined, respectively.

	TTA		**NS**	
	Mean (%)	**SEM (%)**	**Mean (%)**	**SEM (%)**
Mycoplasma	72.9898	5.5536	35.0538	6.9365
Pasteurella	7.6046	3.7231	0.6007	0.4301
Mannheimia	0.7287	0.6186	1.0765	1.0642
Bacteroides	1.8195	1.8044	0.3423	0.0656
Ureaplasma	1.2664	0.6547	0.0867	0.0392
Prevotella	0.4990	0.4545	0.8666	0.2026
Helcococcus	0.3110	0.3089	0.0151	0.0064
Moraxella	0.3292	0.1628	5.9807	4.7562
Fusobacterium	0.2559	0.2431	0.0039	0.0031
Sphingomonas	0.1499	0.0827	2.5698	0.8526
Agrobacterium	0.0905	0.0302	0.5275	0.1772
Porphyromonas	0.1144	0.0998	0.0021	0.0013
Corynebacterium	0.0477	0.0211	1.6248	0.2345
Delftia	0.0920	0.0263	0.1956	0.0525
Parvimonas	0.0582	0.0552	0.0000	0.0000
Campylobacter	0.0508	0.0500	0.0090	0.0067
Pedobacter	0.0258	0.0180	0.3597	0.1445
Coprococcus	0.0279	0.0152	1.0294	0.2634
Methylobacterium	0.0276	0.0131	0.4836	0.1997
Propionibacterium	0.0203	0.0153	0.0373	0.0121
Acinetobacter	0.0285	0.0117	0.8845	0.1718
Sphingobium	0.0228	0.0109	0.2365	0.0950
Ruminobacter	0.0146	0.0101	0.7804	0.2367
Blautia	0.0206	0.0095	1.0208	0.2065
Chryseobacterium	0.0150	0.0104	0.2309	0.1003
Streptococcus	0.0390	0.0295	0.3811	0.1415
Hymenobacter	0.0111	0.0078	0.4047	0.1368
Anaerostipes	0.0097	0.0076	0.1285	0.0418
Ruminococcus	0.0108	0.0063	0.4633	0.0923
[Prevotella]	0.0085	0.0070	0.3363	0.0599
Clostridium	0.0107	0.0061	0.1969	0.0390
Rhodococcus	0.0086	0.0062	0.1323	0.0512
Peptoniphilus	0.0157	0.0149	0.0014	0.0014
Pseudomonas	0.0098	0.0054	0.4204	0.0938
Faecalibacterium	0.0074	0.0054	0.3684	0.0869
Anaerococcus	0.0062	0.0061	0.0042	0.0024

Peptostreptococcus	0.0113	0.0102	0.0000	0.0000
Actinobacillus	0.0162	0.0066	0.0215	0.0128
Acholeplasma	0.0064	0.0045	0.1548	0.0353
Staphylococcus	0.0151	0.0105	0.2336	0.0697
Facklamia	0.0070	0.0032	0.3283	0.0493
Dorea	0.0091	0.0034	0.6892	0.1850
Succinivibrio	0.0062	0.0029	0.7054	0.1908
Butyrivibrio	0.0052	0.0023	0.2820	0.0675
Jeotgalicoccus	0.0050	0.0030	0.2788	0.0460
Psychrobacter	0.0108	0.0052	1.6173	0.6592
Flavobacterium	0.0049	0.0028	0.0692	0.0257
Luteimonas	0.0028	0.0025	0.0606	0.0177
Phascolarctobacterium	0.0037	0.0016	0.1760	0.0453
Trueperella	0.0036	0.0026	0.0059	0.0029
Turicibacter	0.0038	0.0018	0.1945	0.0419
Spirosoma	0.0024	0.0024	0.0152	0.0081
Leptotrichia	0.0102	0.0084	0.0112	0.0105
Micrococcus	0.0029	0.0017	0.0156	0.0063
Arthrobacter	0.0102	0.0076	0.1914	0.0585
Devosia	0.0044	0.0028	0.0665	0.0202
Candidatus Endobugula	0.0019	0.0017	0.1359	0.0292
Proteiniclasticum	0.0025	0.0018	0.0694	0.0259
Treponema	0.0019	0.0016	0.1370	0.0720
Chlamydia	0.0036	0.0036	0.0023	0.0023
Haemophilus	0.0017	0.0015	0.0004	0.0004
Novosphingobium	0.0036	0.0022	0.0023	0.0009
CF231	0.0057	0.0032	0.2383	0.1010
Mycetocola	0.0080	0.0052	0.0624	0.0212
Enhydrobacter	0.0025	0.0011	0.2980	0.1702
Rathayibacter	0.0017	0.0012	0.0448	0.0217
Arcobacter	0.0039	0.0028	0.0159	0.0063
Lactobacillus	0.0053	0.0039	0.1250	0.0489
Bacillus	0.0042	0.0015	0.0209	0.0074
Fibrobacter	0.0030	0.0020	0.0143	0.0069
Sanguibacter	0.0014	0.0013	0.0443	0.0153
Stenotrophomonas	0.0013	0.0013	0.0908	0.0418
Finegoldia	0.0013	0.0013	0.0000	0.0000
Halomonas	0.0012	0.0012	0.0318	0.0081
Anaerovibrio	0.0017	0.0012	0.1349	0.0280
Cloacibacterium	0.0036	0.0020	0.0004	0.0004
5-7N15	0.0024	0.0015	0.1448	0.0557
Planomicrobium	0.0012	0.0008	0.0767	0.0212
Paracoccus	0.0036	0.0026	0.0721	0.0168
Rothia	0.0011	0.0011	0.0023	0.0017
Dyadobacter	0.0027	0.0014	0.0330	0.0125
[Ruminococcus]	0.0010	0.0010	0.0686	0.0147

Granulicatella	0.0013	0.0009	0.0000	0.0000
Sutterella	0.0037	0.0026	0.1628	0.0501
Yaniella	0.0011	0.0008	0.0287	0.0079
Clavibacter	0.0010	0.0009	0.0384	0.0176
Dietzia	0.0055	0.0030	0.0805	0.0199
Janthinobacterium	0.0011	0.0009	0.0112	0.0035
Parabacteroides	0.0010	0.0007	0.0598	0.0162
Selenomonas	0.0010	0.0010	0.0003	0.0003
Aggregatibacter	0.0018	0.0011	1.2588	1.0055
Myroides	0.0023	0.0015	0.1584	0.0569
Aerococcus	0.0026	0.0014	0.1011	0.0256
Microbacterium	0.0009	0.0007	0.0150	0.0046
Cellulomonas	0.0010	0.0006	0.0065	0.0042
Methanobrevibacter	0.0009	0.0005	0.0390	0.0140
Leucobacter	0.0010	0.0006	0.0124	0.0056
Succiniclasticum	0.0011	0.0008	0.0031	0.0017
Cardiobacterium	0.0006	0.0006	0.0000	0.0000
Ochrobactrum	0.0007	0.0005	0.0090	0.0072
Solibacillus	0.0007	0.0005	0.0940	0.0255
Bulleidia	0.0006	0.0005	0.0730	0.0360
Deinococcus	0.0051	0.0040	0.0125	0.0054
[Eubacterium]	0.0005	0.0004	0.0448	0.0150
Brevundimonas	0.0009	0.0006	0.0000	0.0000
Enterococcus	0.0012	0.0009	0.1669	0.0567
Meiothermus	0.0005	0.0005	0.0000	0.0000
Neisseria	0.0012	0.0007	0.0245	0.0154
Rhizobium	0.0014	0.0008	0.0054	0.0031
Bifidobacterium	0.0007	0.0003	0.0538	0.0108
Chroococcidiopsis	0.0005	0.0005	0.0000	0.0000
Kineococcus	0.0005	0.0005	0.0378	0.0181
Sediminibacterium	0.0008	0.0004	0.0002	0.0002
Ornithobacterium	0.0004	0.0004	0.0119	0.0063
Pseudoxanthomonas	0.0004	0.0004	0.0000	0.0000
Rummeliibacillus	0.0008	0.0004	0.0454	0.0126
Brachybacterium	0.0025	0.0014	0.0653	0.0177
Erwinia	0.0012	0.0007	0.1595	0.0878
Wautersiella	0.0012	0.0012	0.0623	0.0203
Coprobacillus	0.0016	0.0013	0.0210	0.0078
Trichococcus	0.0005	0.0002	0.0863	0.0204
Veillonella	0.0005	0.0003	0.0123	0.0119
Cellvibrio	0.0019	0.0019	0.0381	0.0118
Comamonas	0.0003	0.0002	0.0112	0.0045
Erysipelothrix	0.0003	0.0002	0.0729	0.0181
Filifactor	0.0007	0.0007	0.0000	0.0000
Oscillospira	0.0006	0.0004	0.2663	0.0608
Salinicoccus	0.0003	0.0003	0.0053	0.0026

vadinCA11	0.0002	0.0002	0.0000	0.0000
BD2-13	0.0003	0.0002	0.0214	0.0119
Guggenheimella	0.0003	0.0002	0.0279	0.0106
Shuttleworthia	0.0003	0.0003	0.0137	0.0050
Akkermansia	0.0006	0.0006	0.0163	0.0074
Anaeroplasma	0.0003	0.0002	0.0459	0.0247
Kocuria	0.0007	0.0006	0.0090	0.0045
Rhodobacter	0.0015	0.0013	0.0355	0.0197
Roseburia	0.0018	0.0017	0.0316	0.0142
Streptomyces	0.0002	0.0002	0.0321	0.0116
Tissierella	0.0004	0.0004	0.0130	0.0056
Actinomyces	0.0001	0.0001	0.0007	0.0007
Aeromicrobium	0.0001	0.0001	0.0275	0.0118
Cryocola	0.0001	0.0001	0.0024	0.0014
Morganella	0.0002	0.0002	0.0003	0.0003
Mycobacterium	0.0002	0.0001	0.0036	0.0015
p-75-a5	0.0002	0.0002	0.0043	0.0018
Sphingobacterium	0.0002	0.0002	0.0714	0.0280
Achromobacter	0.0001	0.0001	0.0000	0.0000
Agrococcus	0.0001	0.0001	0.0034	0.0015
Bibersteinia	0.0002	0.0002	0.0035	0.0030
Capnocytophaga	0.0001	0.0001	0.0000	0.0000
Luteococcus	0.0001	0.0001	0.0010	0.0010
Mesorhizobium	0.0002	0.0002	0.0000	0.0000
Natronobacillus	0.0001	0.0001	0.0098	0.0049
Nevskia	0.0002	0.0002	0.0003	0.0003
Ralstonia	0.0001	0.0001	0.0032	0.0031
RFN20	0.0001	0.0001	0.0190	0.0096
Rhodanobacter	0.0002	0.0002	0.0000	0.0000
Rhodoplanes	0.0002	0.0002	0.0023	0.0023
Saccharopolyspora	0.0001	0.0001	0.0487	0.0246
Tepidimonas	0.0002	0.0002	0.0000	0.0000
Tindallia	0.0001	0.0001	0.0011	0.0008
Varibaculum	0.0002	0.0002	0.0000	0.0000
YRC22	0.0002	0.0002	0.0027	0.0016
Brevibacterium	0.0003	0.0003	0.0249	0.0084
Cupriavidus	0.0001	0.0001	0.0011	0.0011
Curtobacterium	0.0000	0.0000	0.0010	0.0007
Demequina	0.0000	0.0000	0.0153	0.0098
GW-34	0.0001	0.0001	0.0233	0.0078
Lactococcus	0.0013	0.0013	0.0013	0.0007
Lautropia	0.0000	0.0000	0.0013	0.0008
Leuconostoc	0.0013	0.0013	0.0054	0.0039
Marinobacter	0.0001	0.0001	0.0112	0.0051
Patulibacter	0.0001	0.0001	0.0043	0.0041
Peptococcus	0.0001	0.0001	0.0032	0.0012

Propionivibrio	0.0001	0.0001	0.0007	0.0007
Pseudoclavibacter	0.0000	0.0000	0.0336	0.0110
Rheinheimera	0.0001	0.0001	0.0064	0.0037
Rudanella	0.0001	0.0001	0.0002	0.0002
Sharpea	0.0000	0.0000	0.0160	0.0124
Sporosarcina	0.0001	0.0001	0.0093	0.0047
Thauera	0.0003	0.0003	0.0048	0.0032
Vagococcus	0.0003	0.0003	0.0060	0.0045
24838	0.0000	0.0000	0.0035	0.0035
Acetobacter	0.0000	0.0000	0.0013	0.0013
Acetobacterium	0.0000	0.0000	0.0025	0.0025
Acidaminococcus	0.0000	0.0000	0.0006	0.0006
Acidovorax	0.0000	0.0000	0.0096	0.0052
Adlercreutzia	0.0000	0.0000	0.0031	0.0019
Aequorivita	0.0000	0.0000	0.0165	0.0046
Alcanivorax	0.0000	0.0000	0.0020	0.0015
Alkalibacter	0.0000	0.0000	0.0016	0.0016
Alkalibacterium	0.0000	0.0000	0.0064	0.0027
Alkanindiges	0.0000	0.0000	0.0004	0.0004
Anaerofilum	0.0000	0.0000	0.0002	0.0002
Anaerolinea	0.0000	0.0000	0.0034	0.0034
Anaerospora	0.0000	0.0000	0.0194	0.0117
Arsenicicoccus	0.0000	0.0000	0.0019	0.0019
Atopobium	0.0000	0.0000	0.0052	0.0025
B-42	0.0000	0.0000	0.0116	0.0065
Bdellovibrio	0.0000	0.0000	0.0084	0.0039
BF311	0.0000	0.0000	0.0005	0.0005
Brumimicrobium	0.0000	0.0000	0.0247	0.0099
Caldilinea	0.0000	0.0000	0.0023	0.0023
Candidatus Arthromitus	0.0000	0.0000	0.0017	0.0017
Candidatus Portiera	0.0000	0.0000	0.0178	0.0078
Carnobacterium	0.0000	0.0000	0.0014	0.0009
Catenibacterium	0.0000	0.0000	0.0021	0.0021
Chelonobacter	0.0000	0.0000	0.0002	0.0002
Chitinophaga	0.0000	0.0000	0.0091	0.0091
Collinsella	0.0000	0.0000	0.0024	0.0015
Cryomorpha	0.0000	0.0000	0.0011	0.0011
Cytophaga	0.0000	0.0000	0.0023	0.0023
Dermabacter	0.0000	0.0000	0.0008	0.0005
Desemzia	0.0000	0.0000	0.0052	0.0038
Desulfobulbus	0.0000	0.0000	0.0027	0.0016
Desulfovibrio	0.0000	0.0000	0.0006	0.0006
Dialister	0.0000	0.0000	0.0002	0.0002
Dokdonella	0.0000	0.0000	0.0042	0.0042
Dysgonomonas	0.0000	0.0000	0.0027	0.0018
Ellin506	0.0000	0.0000	0.0016	0.0016

Enterobacter	0.0000	0.0000	0.0009	0.0009
Epulopiscium	0.0000	0.0000	0.0272	0.0205
Euzebya	0.0000	0.0000	0.0003	0.0003
Flavisolibacter	0.0000	0.0000	0.0013	0.0007
Flectobacillus	0.0000	0.0000	0.0019	0.0019
Fluviicola	0.0000	0.0000	0.0123	0.0052
Friedmanniella	0.0000	0.0000	0.0014	0.0013
Gallicola	0.0000	0.0000	0.0148	0.0105
Gelidibacter	0.0000	0.0000	0.0151	0.0086
Georgenia	0.0000	0.0000	0.0086	0.0030
Gordonia	0.0000	0.0000	0.0025	0.0017
HTCC	0.0000	0.0000	0.0009	0.0009
Hydrogenophaga	0.0000	0.0000	0.0015	0.0008
Hylemonella	0.0000	0.0000	0.0021	0.0021
Jonesia	0.0000	0.0000	0.0041	0.0025
Kaistobacter	0.0000	0.0000	0.0033	0.0022
Klebsiella	0.0000	0.0000	0.0014	0.0014
Kurthia	0.0000	0.0000	0.0041	0.0027
Labrys	0.0000	0.0000	0.0030	0.0030
Lachnobacterium	0.0000	0.0000	0.0402	0.0125
Lachnospira	0.0000	0.0000	0.0106	0.0062
Leadbetterella	0.0000	0.0000	0.0011	0.0011
Legionella	0.0000	0.0000	0.0006	0.0006
Luteibacter	0.0000	0.0000	0.0033	0.0026
Luteolibacter	0.0000	0.0000	0.0018	0.0018
Lysinibacillus	0.0000	0.0000	0.0033	0.0017
Marinilactibacillus	0.0000	0.0000	0.0034	0.0034
Marinococcus	0.0000	0.0000	0.0015	0.0013
Megamonas	0.0000	0.0000	0.0008	0.0006
Megasphaera	0.0000	0.0000	0.0051	0.0029
Methanosphaera	0.0000	0.0000	0.0045	0.0023
Methylibium	0.0000	0.0000	0.0018	0.0018
Methylophaga	0.0000	0.0000	0.0119	0.0077
Methylotenera	0.0000	0.0000	0.0018	0.0018
Microbispora	0.0000	0.0000	0.0023	0.0016
Mitsuokella	0.0000	0.0000	0.0008	0.0008
Mogibacterium	0.0000	0.0000	0.0167	0.0050
Moryella	0.0000	0.0000	0.0030	0.0030
ND137	0.0000	0.0000	0.0009	0.0009
Nesterenkonia	0.0000	0.0000	0.0086	0.0046
Niigata-25	0.0000	0.0000	0.0006	0.0006
Nitratireductor	0.0000	0.0000	0.0021	0.0021
Nocardioides	0.0000	0.0000	0.0065	0.0044
Nocardiopsis	0.0000	0.0000	0.0024	0.0016
Odoribacter	0.0000	0.0000	0.0071	0.0042
Oleibacter	0.0000	0.0000	0.0031	0.0021

Oligella	0.0000	0.0000	0.0515	0.0197
Olivibacter	0.0000	0.0000	0.0066	0.0046
Paenibacillus	0.0000	0.0000	0.0107	0.0037
Paludibacter	0.0000	0.0000	0.0163	0.0057
Pantoea	0.0000	0.0000	0.0002	0.0002
Paraprevotella	0.0000	0.0000	0.0002	0.0002
ph2	0.0000	0.0000	0.0003	0.0003
Phycicoccus	0.0000	0.0000	0.0007	0.0005
Phyllobacterium	0.0000	0.0000	0.0009	0.0009
Pigmentiphaga	0.0000	0.0000	0.0054	0.0035
Planctomyces	0.0000	0.0000	0.0022	0.0016
Prauserella	0.0000	0.0000	0.0230	0.0116
Propionicimonas	0.0000	0.0000	0.0227	0.0085
Pseudidiomarina	0.0000	0.0000	0.0038	0.0017
Pseudoalteromonas	0.0000	0.0000	0.0006	0.0004
Pseudonocardia	0.0000	0.0000	0.0002	0.0002
Pseudoramibacter	0.0000	0.0000	0.0034	0.0027
Pyramidobacter	0.0000	0.0000	0.0022	0.0016
rc4-4	0.0000	0.0000	0.0139	0.0062
Saccharomonospora	0.0000	0.0000	0.0003	0.0003
Salana	0.0000	0.0000	0.0020	0.0012
Sedimentibacter	0.0000	0.0000	0.0172	0.0061
Skermanella	0.0000	0.0000	0.0009	0.0009
SMB53	0.0000	0.0000	0.0012	0.0010
Sphaerochaeta	0.0000	0.0000	0.0044	0.0032
Terracoccus	0.0000	0.0000	0.0022	0.0018
Tessaracoccus	0.0000	0.0000	0.0214	0.0056
Variovorax	0.0000	0.0000	0.0001	0.0001
Vibrio	0.0000	0.0000	0.0101	0.0057
Vitreoscilla	0.0000	0.0000	0.0014	0.0014
Vogesella	0.0000	0.0000	0.0025	0.0025
W22	0.0000	0.0000	0.0015	0.0011
Weeksella	0.0000	0.0000	0.0177	0.0088
Weissella	0.0000	0.0000	0.0014	0.0014
Williamsia	0.0000	0.0000	0.0075	0.0050
Xanthobacter	0.0000	0.0000	0.0076	0.0076
Xylanimicrobium	0.0000	0.0000	0.0084	0.0033
Zhouia	0.0000	0.0000	0.0024	0.0016
Zoogloea	0.0000	0.0000	0.0008	0.0008

4038 **Table S2**. Operational taxonomic units (OTUs) identified at the species level in the nasal swab (NS)
4039 and trans-tracheal aspiration (TTA) samples. Data are reported as average relative abundance and
4040 standard error of the mean (SEM).

	TTA		NS	
	Mean (%)	**SEM (%)**	**Mean (%)**	**SEM (%)**
Pasteurella multocida	7.6043	3.7230	0.6006	0.4301
Porphyromonas endodontalis	0.1004	0.1002	0.0000	0.0000
Propionibacterium acnes	0.0203	0.0153	0.0373	0.0121
Methylobacterium adhaesivum	0.0188	0.0128	0.3689	0.1526
Prevotella copri	0.0157	0.0114	0.4865	0.1269
Acinetobacter lwoffii	0.0132	0.0051	0.4644	0.0917
Rhodococcus fascians	0.0078	0.0057	0.1203	0.0485
Psychrobacter sanguinis	0.0077	0.0031	1.5771	0.6589
Faecalibacterium prausnitzii	0.0074	0.0054	0.3684	0.0869
Staphylococcus equorum	0.0063	0.0051	0.0443	0.0128
Actinobacillus capsulatus	0.0063	0.0027	0.0007	0.0007
Staphylococcus sciuri	0.0054	0.0051	0.0641	0.0286
Jeotgalicoccus psychrophilus	0.0048	0.0029	0.2165	0.0408
Blautia producta	0.0047	0.0028	0.2376	0.0563
Bacteroides eggerthii	0.0047	0.0038	0.0000	0.0000
Arcobacter cryaerophilus	0.0039	0.0028	0.0104	0.0041
Fibrobacter succinogenes	0.0030	0.0020	0.0143	0.0069
Clostridium neonatale	0.0028	0.0020	0.0107	0.0042
Brachybacterium conglomeratum	0.0025	0.0014	0.0636	0.0175
Sphingomonas yabuuchiae	0.0024	0.0014	0.0029	0.0020
Sphingomonas wittichii	0.0024	0.0016	0.0325	0.0184
Pedobacter cryoconitis	0.0021	0.0016	0.0464	0.0296
Novosphingobium capsulatum	0.0019	0.0015	0.0000	0.0000
Pseudomonas fragi	0.0017	0.0013	0.0012	0.0007
Rathayibacter caricis	0.0017	0.0012	0.0448	0.0217
Prevotella stercorea	0.0016	0.0010	0.1500	0.0332
Acinetobacter johnsonii	0.0016	0.0013	0.0307	0.0114
Haemophilus parainfluenzae	0.0015	0.0015	0.0004	0.0004
Rhizobium leguminosarum	0.0014	0.0008	0.0054	0.0031
Pseudomonas viridiflava	0.0013	0.0009	0.0909	0.0394
Rothia dentocariosa	0.0011	0.0011	0.0016	0.0016

Ruminococcus bromii	0.0010	0.0010	0.0010	0.0009
Sphingomonas echinoides	0.0010	0.0009	0.0192	0.0086
Brevundimonas diminuta	0.0009	0.0006	0.0000	0.0000
Paracoccus marcusii	0.0008	0.0008	0.0114	0.0053
Myroides odoratimimus	0.0008	0.0007	0.1118	0.0371
Kocuria rhizophila	0.0007	0.0006	0.0090	0.0045
Pseudomonas stutzeri	0.0005	0.0005	0.0060	0.0043
[Eubacterium] biforme	0.0005	0.0004	0.0316	0.0099
Veillonella dispar	0.0005	0.0003	0.0123	0.0119
Acholeplasma Laidlawii	0.0005	0.0004	0.0326	0.0106
Bacillus Cereus	0.0004	0.0002	0.0021	0.0007
Bacteroides coprophilus	0.0003	0.0002	0.0326	0.0078
Lactobacillus Brevis	0.0003	0.0003	0.0047	0.0028
Acinetobacter Schindleri	0.0003	0.0003	0.0005	0.0005
Corynebacterium Variabile	0.0003	0.0003	0.0141	0.0079
Morganella Morganii	0.0002	0.0002	0.0003	0.0003
Bacteroides Barnesiae	0.0002	0.0002	0.0000	0.0000
Bifidobacterium pseudolongum	0.0001	0.0001	0.0235	0.0052
Bulleidia p-1630-c5	0.0001	0.0001	0.0511	0.0341
Prevotella intermedia	0.0001	0.0001	0.0000	0.0000
Bacillus flexus	0.0001	0.0001	0.0000	0.0000
Roseburia faecis	0.0001	0.0001	0.0008	0.0004
Pseudoclavibacter bifida	0.0000	0.0000	0.0336	0.0110
Sharpea p-3329-23G2	0.0000	0.0000	0.0147	0.0117
[Eubacterium] cylindroides	0.0000	0.0000	0.0034	0.0024
[Eubacterium] dolichum	0.0000	0.0000	0.0097	0.0052
[Ruminococcus] gnavus	0.0000	0.0000	0.0006	0.0004
Agrococcus jenensis	0.0000	0.0000	0.0001	0.0001
Akkermansia muciniphila	0.0000	0.0000	0.0013	0.0013
Bacillus thermoamylovorans	0.0000	0.0000	0.0013	0.0013
Bacteroides fragilis	0.0000	0.0000	0.0004	0.0004
Bacteroides plebeius	0.0000	0.0000	0.0068	0.0026
Bacteroides uniformis	0.0000	0.0000	0.0028	0.0023
Bifidobacterium longum	0.0000	0.0000	0.0072	0.0037
Chelonobacter Taxon	0.0000	0.0000	0.0002	0.0002
Clostridium hiranonis	0.0000	0.0000	0.0001	0.0001
Clostridium perfringens	0.0000	0.0000	0.0008	0.0008

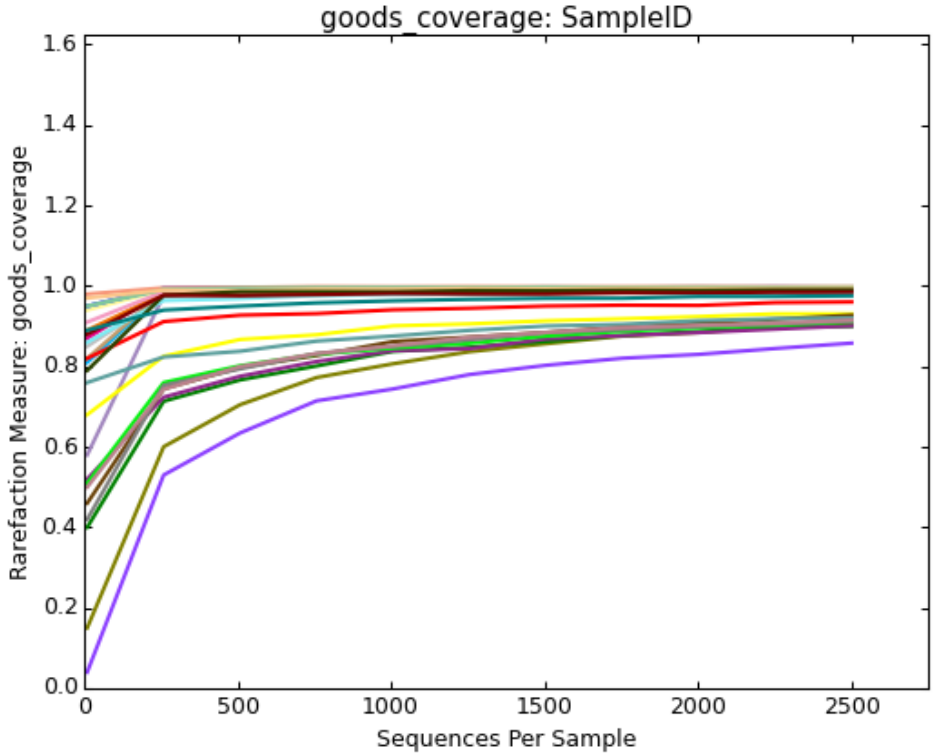
Collinsella aerofaciens	0.0000	0.0000	0.0023	0.0015
Coprococcus eutactus	0.0000	0.0000	0.0095	0.0054
Corynebacterium pilosum	0.0000	0.0000	0.0030	0.0017
Deinococcus aquatilis	0.0000	0.0000	0.0008	0.0008
Enterococcus cecorum	0.0000	0.0000	0.0046	0.0046
Flavobacterium succinicans	0.0000	0.0000	0.0016	0.0014
Janthinobacterium lividum	0.0000	0.0000	0.0002	0.0002
Lactobacillus agilis	0.0000	0.0000	0.0008	0.0008
Lactobacillus reuteri	0.0000	0.0000	0.0004	0.0004
Lactobacillus ruminis	0.0000	0.0000	0.0033	0.0024
Luteibacter rhizovicinus	0.0000	0.0000	0.0033	0.0026
Lysinibacillus boronitolerans	0.0000	0.0000	0.0033	0.0017
Marinilactibacillus psychrotolerans	0.0000	0.0000	0.0034	0.0034
Methylotenera mobilis	0.0000	0.0000	0.0018	0.0018
Microbispora rosea	0.0000	0.0000	0.0023	0.0016
Nocardioides plantarum	0.0000	0.0000	0.0010	0.0010
Nocardiopsis exhalans	0.0000	0.0000	0.0024	0.0016
Ochrobactrum intermedium	0.0000	0.0000	0.0006	0.0004
Pantoea agglomerans	0.0000	0.0000	0.0002	0.0002
Prauserella rugosa	0.0000	0.0000	0.0230	0.0116
Psychrobacter pacificensis	0.0000	0.0000	0.0024	0.0019
Rothia aeria	0.0000	0.0000	0.0008	0.0008
Ruminococcus flavefaciens	0.0000	0.0000	0.0085	0.0036
Saccharopolyspora hirsuta	0.0000	0.0000	0.0033	0.0020
Salana multivorans	0.0000	0.0000	0.0020	0.0012
Selenomonas ruminantium	0.0000	0.0000	0.0003	0.0003
Sphingobacterium faecium	0.0000	0.0000	0.0063	0.0022
Sphingobacterium mizutaii	0.0000	0.0000	0.0056	0.0031
Sphingobacterium multivorum	0.0000	0.0000	0.0030	0.0023
Streptococcus minor	0.0000	0.0000	0.0081	0.0049
Vibrio metschnikovii	0.0000	0.0000	0.0069	0.0041
Vibrio rumoiensis	0.0000	0.0000	0.0012	0.0012

APPENDIX 2

Table S3. Alpha diversity metrics of each sample. The alpha diversity metric presented include the Good's coverage, Chao1 and Observed species indices which estimate the species richness; Simpson and Shannon indices which estimate species evenness and Phylogenetic Diversity (PD) whole tree index which estimate the species diversity.

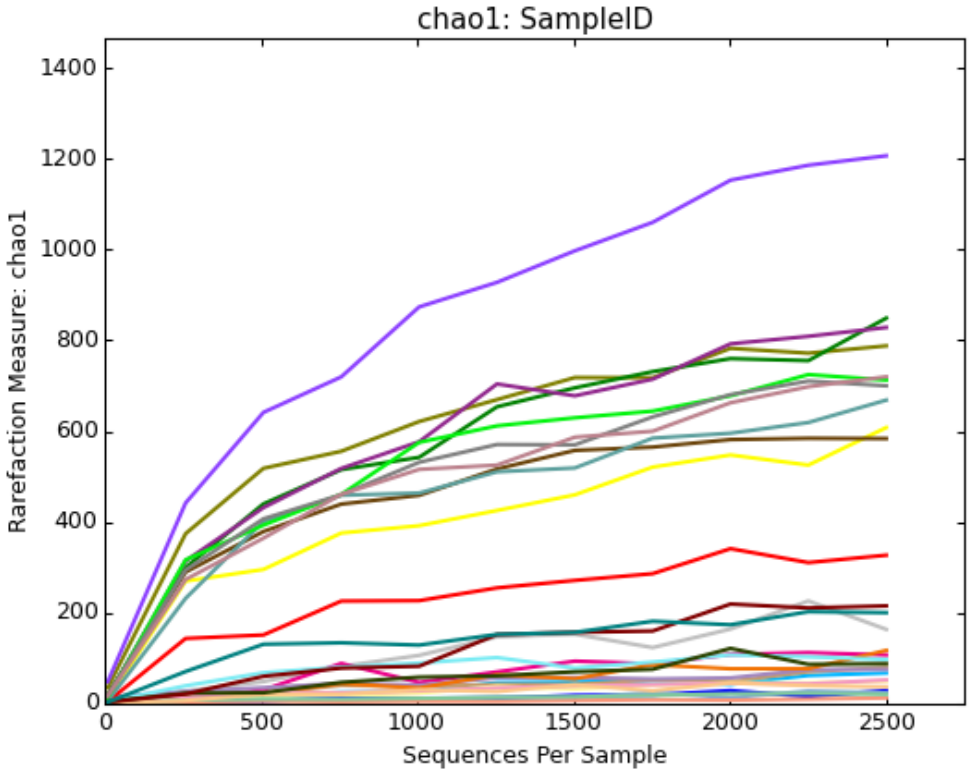
Sample_ID	Good's coverage	Chao1	Observed species	Simpson	Shannon	PD whole tree
4_TTA	98.8%	97.6	56.0	0.73	2.33	8.1
5_TTA	99.5%	37.5	19.5	0.06	0.27	4.4
3_TTA	99.8%	20.9	14.0	0.53	1.24	3.5
2_TTA	99.5%	52.5	25.2	0.48	1.54	5.2
21_TTA	99.1%	88.2	40.6	0.40	1.35	6.0
15_TTA	98.5%	163.9	52.6	0.29	1.09	9.2
16_TTA	98.9%	107.6	36.8	0.58	1.67	7.4
17_TTA	99.4%	67.8	25.6	0.71	2.15	5.9
10_TTA	99.8%	12.9	7.7	0.03	0.15	2.1
6_TTA	96.0%	327.2	155.7	0.59	2.27	18.0
7_TTA	99.7%	28.9	12.2	0.25	0.68	3.6
8_TTA	99.1%	117.5	34.1	0.16	0.67	6.4
13_TTA	99.7%	24.2	13.3	0.19	0.62	3.6
1_TTA	99.4%	75.6	44.6	0.86	3.42	5.7
14_TTA	99.5%	50.1	22.1	0.55	1.34	4.4
18_TTA	99.3%	80.1	31.2	0.57	1.90	6.3
20_TTA	97.6%	200.7	99.2	0.64	2.17	11.4
15_NS	90.8%	788.1	540.8	0.98	7.53	38.2
16_NS	85.8%	1206.6	697.2	0.99	8.31	45.2
10_NS	92.0%	668.6	315.3	0.67	3.53	28.4
11_NS	91.1%	712.8	392.9	0.87	5.12	32.2
6_NS	90.0%	849.1	459.2	0.93	6.10	34.8
7_NS	90.1%	828.6	436.1	0.84	5.30	33.2
8_NS	93.1%	608.1	270.6	0.64	3.34	24.9
12_NS	92.7%	584.0	373.4	0.89	5.28	30.3
13_NS	91.3%	700.1	390.0	0.91	5.35	31.4
18_NS	91.5%	720.0	390.2	0.71	4.60	28.5
20_NS	98.5%	215.6	53.3	0.62	1.93	8.5

4047 **Fig. S1. Rarefaction curves for each sample for the Good's coverage indices.**



4048

4049 **Fig. S2. Rarefaction curves for each sample for the Chao1 indices.**



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4051

Fig. S3. Rarefaction curves for each sample for the Observed species indices.

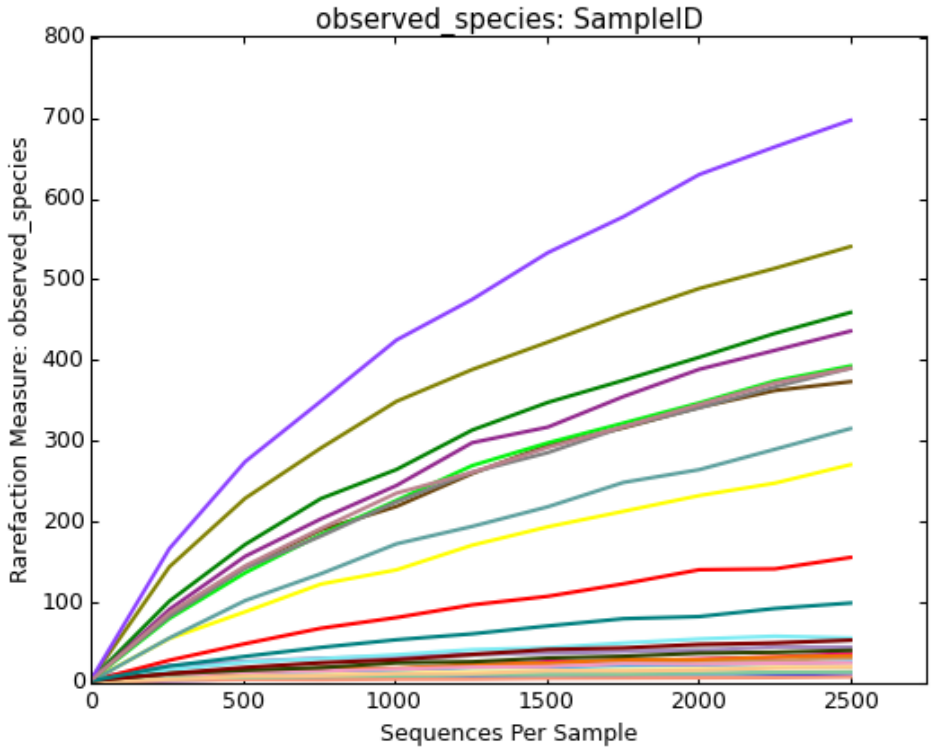


Fig. S4. Rarefaction curves for each sample for the Simpson indices.

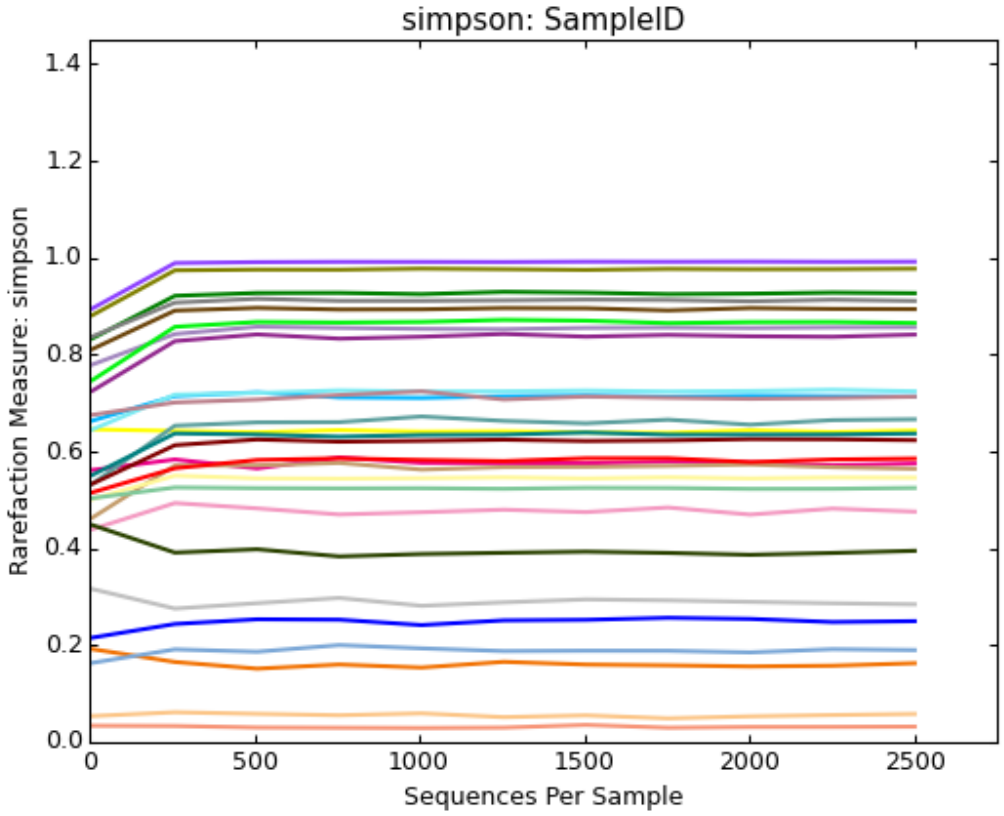


Fig. S5. Rarefaction curves for each sample for the Shannon indices.

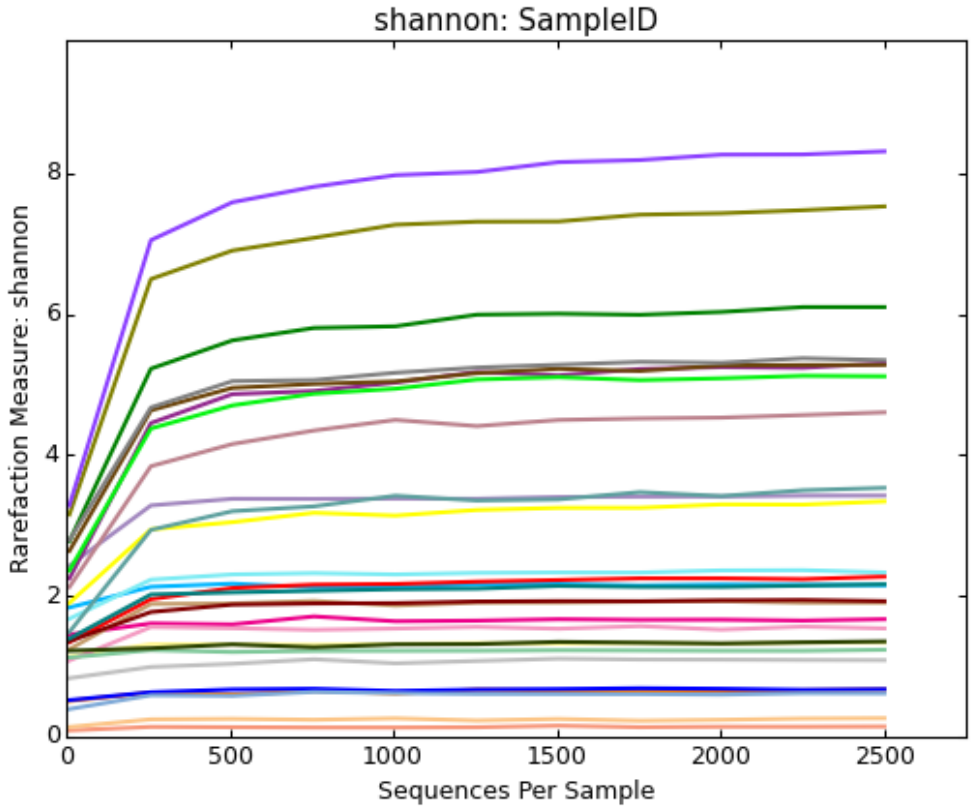
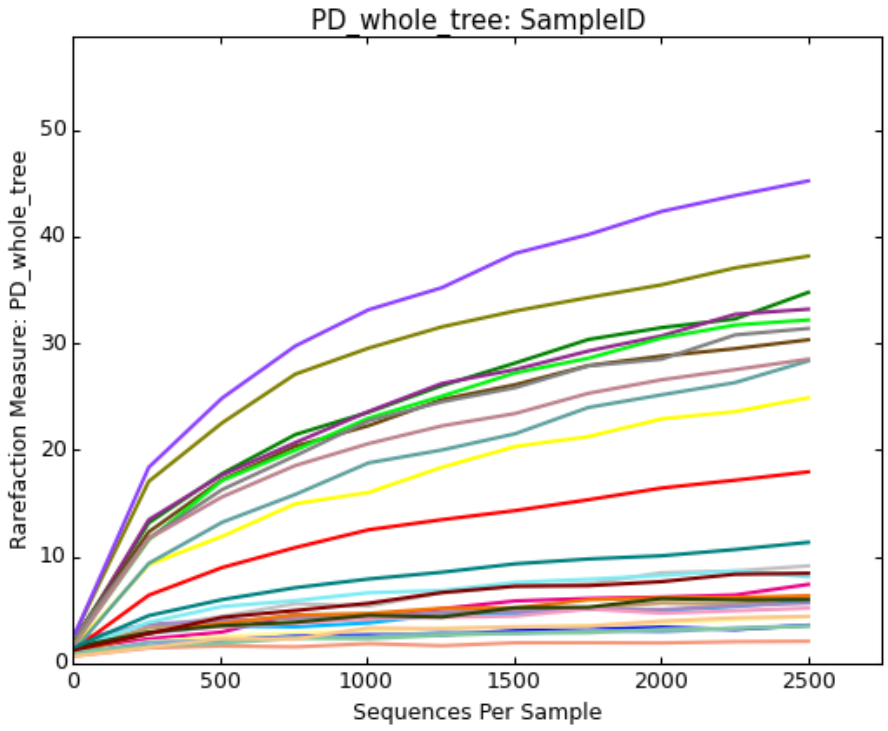


Fig. S6. Rarefaction curves for each sample for the Phylogenetic diversity whole tree indices.



APPENDIX 3

Table S4. Table reporting the differential abundances of taxa, found in the nasal swabs (NS = 11) and in the trans-tracheal aspiration (TTA = 17) samples obtained by the DESeq2 analysis. As reported in the DESeq2 support information, the "base mean" column reports the mean of normalized counts for all samples, while the "log2FoldChange" column reports the log fold change calculated for the TTA as compared to the NS samples, with the relative standard error in the adjacent column "lfcSE" (that is log2 fold change Standard Error). For statistical significance, *P* values are reported without (*P*value) and with adjustment (padj) for multiple testing with the false discovery rate (FDR).

Phylum	Class	Order	Family	Genus	Species	Base mean	Log2Fold Change	lfcSE	Stat	*P* value	p adj
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira		21.4	-5.0	0.5	-9.4	4.7e-21	1.9e-18
Firmicutes	Clostridia	Clostridiales	Other	Other	Other	61.3	-5.0	0.6	-8.1	6.4e-16	1.3e-13
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae			21.2	-4.3	0.5	-7.9	3.0e-15	4.1e-13
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	sanguinis	108.4	-5.8	0.8	-7.4	1.4e-13	1.4e-11
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae			23.0	-3.9	0.5	-7.3	3.7e-13	3.0e-11
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae			9.4	-3.8	0.5	-7.2	4.8e-13	3.1e-11
TM7	TM7-3					7.1	-3.5	0.5	-7.2	5.4e-13	3.1e-11
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	Other	8.1	-3.6	0.5	-7.1	1.4e-12	6.8e-11
Cyanobacteria	Chloroplast	Streptophyta				22.6	-4.5	0.6	-7.1	1.7e-12	7.7e-11
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Trichococcus		9.0	-3.6	0.5	-7.0	3.1e-12	1.2e-10
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus		13.7	-4.1	0.6	-6.9	6.8e-12	2.3e-10
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae			8.5	-3.4	0.5	-6.9	6.3e-12	2.3e-10
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae			70.3	5.9	0.9	6.8	1.1e-11	3.3e-10
Firmicutes	Clostridia	Clostridiales				174.8	-4.3	0.6	-6.6	3.6e-11	1.0e-09
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter		28.4	-4.2	0.6	-6.6	4.5e-11	1.2e-09
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas		195.1	-4.2	0.6	-6.5	7.0e-11	1.8e-09
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio		60.8	-4.5	0.7	-6.5	7.9e-11	1.9e-09
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae			60.8	-4.1	0.6	-6.4	1.2e-10	2.7e-09
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae			8.8	-3.7	0.6	-6.4	1.6e-10	3.3e-09
Firmicutes	Bacilli	Bacillales	Planococcaceae	Solibacillus		9.6	-3.4	0.5	-6.4	1.7e-10	3.5e-09

Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Erysipelothrix		6.3	-3.1	0.5	-6.2	4.9e-10	9.4e-09
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea		41.6	-3.8	0.6	-6.2	6.2e-10	1.1e-08
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium	conglomeratum	6.2	-2.9	0.5	-6.2	7.4e-10	1.3e-08
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Other	Other	165.8	4.9	0.8	6.1	1.4e-09	2.4e-08
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter		17.3	-4.0	0.7	-6.0	1.8e-09	2.9e-08
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella		15.5	-3.8	0.6	-6.0	1.9e-09	2.9e-08
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Oligella		5.2	-3.2	0.5	-6.0	2.2e-09	3.2e-08
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Myroides	odoratimimus	7.7	-3.4	0.6	-5.9	2.9e-09	4.1e-08
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	adhaesivum	30.9	-3.9	0.7	-5.9	3.0e-09	4.1e-08
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter		26.3	-4.3	0.7	-5.9	3.1e-09	4.1e-08
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus		6.4	-3.1	0.5	-5.9	3.3e-09	4.3e-08
Proteobacteria	Alphaproteobacteria	Rickettsiales	mitochondria	Other	Other	7.0	-3.5	0.6	-5.9	4.2e-09	5.2e-08
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	producta	16.1	-3.3	0.6	-5.8	5.8e-09	7.0e-08
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae			26.0	-3.6	0.6	-5.8	6.5e-09	7.4e-08
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae			30.3	-3.9	0.7	-5.8	6.3e-09	7.4e-08
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes		9.9	-3.1	0.5	-5.8	6.8e-09	7.5e-08
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus		9.5	-3.1	0.5	-5.8	7.0e-09	7.6e-08
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	CF231		16.6	-3.4	0.6	-5.8	8.3e-09	8.8e-08
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae			6.6	-3.1	0.6	-5.7	1.3e-08	1.3e-07
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	stercorea	15.7	-3.5	0.6	-5.7	1.3e-08	1.3e-07
TM7	TM7-3	CW040	F16			9.6	-3.0	0.5	-5.7	1.4e-08	1.3e-07
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae			12.8	-3.2	0.6	-5.7	1.6e-08	1.5e-07
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus		6.0	-3.2	0.6	-5.6	1.9e-08	1.8e-07
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Ureaplasma		306.6	5.4	1.0	5.6	2.6e-08	2.4e-07
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium		5,9	-3.2	0.6	-5.6	2.8e-08	2.8e-08
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	GW-34		4.7	-3.0	0.5	-5.6	2.8e-08	2.5e-07
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Wautersiella		6.5	-2.9	0.5	-5.4	6.0e-08	5.1e-07
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter		18.6	-3.1	0.6	-5.4	6.4e-08	5.3e-07
Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae			4.9	-3.1	0.6	-5.4	6.8e-08	5.5e-07

Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Anaerovibrio		11.4	-3.2	0.6	-5.4	6.9e-08	5.6e-07
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae			11.0	-3.3	0.6	-5.4	8.1e-08	6.4e-07
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnobacterium		4.3	-2.9	0.5	-5.3	9.1e-08	6.8e-07
Proteobacteria	Alphaproteobacteria	Rhizobiales	Aurantimonadaceae			11.1	-3.1	0.6	-5.3	9.2e-08	6.8e-07
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae			4.4	-3.0	0.6	-5.3	8.9e-08	6.8e-07
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	5-7N15		11.4	-3.2	0.6	-5.3	9.6e-08	7.0e-07
Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	Turicibacter		20.3	-3.3	0.6	-5.3	1.0e-07	7.3e-07
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae			6.8	-2.9	0.5	-5.3	1.5e-07	1.0e-06
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Pseudoclavibacter	bifida	3.8	-2.6	0.5	-5.2	2.0e-07	1.4e-06
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae			5.5	-3.2	0.6	-5.2	2.0e-07	1.4e-06
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia		6.9	-3.1	0.6	-5.1	2.7e-07	1.8e-06
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Other	Other	43.1	-4.1	0.8	-5.1	3.0e-07	2.0e-06
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia		30.8	-3.3	0.6	-5.1	3.3e-07	2.1e-06
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides		29.3	-3.0	0.6	-5.1	3.7e-07	2.3e-06
Bacteroidetes	Bacteroidia	Bacteroidales	RF16			13.4	-3.0	0.6	-5.1	4.3e-07	2.6e-06
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Other	Other	5.0	-2.4	0.5	-5.1	4.3e-07	2.6e-06
Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae			5.9	-2.7	0.5	-5.1	4.4e-07	2.6e-06
Firmicutes	Bacilli	Bacillales	Planococcaceae	Rummeliibacillus		6.0	-2.8	0.6	-5.0	4.9e-07	2.9e-06
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	Dietzia		7.7	-2.6	0.5	-5.0	5.8e-07	3.4e-06
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio		23.9	-3.1	0.6	-5.0	6.5e-07	3.8e-06
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae			296.9	-3.7	0.8	-4.9	7.5e-07	4.3e-06
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	fascians	10.5	-3.1	0.6	-4.9	9.1e-07	5.2e-06
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus		13.4	-3.2	0.7	-4.9	9.6e-07	5.4e-06
Bacteroidetes	Bacteroidia	Bacteroidales				26.8	-3.0	0.6	-4.9	1.0e-06	5.6e-06
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Cellvibrio		4.4	-2.5	0.5	-4.9	1.0e-06	5.6e-06
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas		4,2	-2.7	0.5	-4.9	1.0e-06	1.0e-06
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	sciuri	5.5	-2.7	0.6	-4.9	1.1e-06	5.8e-06
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma		4.6	-2.7	0.6	-4.9	1.2e-06	6.3e-06
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Tessaracoccus		3.4	-2.5	0.5	-4.8	1.3e-06	6.7e-06

Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae			11.7	-2.8	0.6	-4.8	1.4e-06	6.9e-06
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae			220.9	-3.7	0.8	-4.8	1.5e-06	7.5e-06
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus	psychrophilus	22.8	-3.2	0.7	-4.8	1.6e-06	8.0e-06
Cyanobacteria	4C0d-2	YS2				5.0	-2.8	0.6	-4.8	1.7e-06	8.4e-06
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas		30.2	-3.2	0.7	-4.8	1.8e-06	8.8e-06
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	lwoffii	44.4	-3.2	0.7	-4.8	2.0e-06	9.5e-06
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Candidatus Endobugula		12,6	-2.9	0.6	-4.7	2.1e-06	2.1e-06
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Phascolarctobacterium		19,2	-3.1	0.6	-4.7	2.2e-06	2.2e-06
Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium		3.2	-2.3	0.5	-4.7	2.7e-06	1.2e-05
Bacteroidetes	Bacteroidia	Bacteroidales	S24-7			15.8	-2.9	0.6	-4.7	2.8e-06	1.3e-05
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	viridiflava	8.7	-2.8	0.6	-4.7	3.0e-06	1.4e-05
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella	multocida	1814.2	5.1	1.1	4.6	3.4e-06	1.5e-05
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus		4.0	-2.8	0.6	-4.6	3.6e-06	1.6e-05
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae			14.8	-2.9	0.6	-4.6	4.4e-06	1.9e-05
Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	Guggenheimella		4.7	-2.6	0.6	-4.6	4.5e-06	1.9e-05
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides		6.9	-2.5	0.5	-4.6	5.2e-06	2.2e-05
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	laidlawii	4.0	-2.4	0.5	-4.5	5.4e-06	2.3e-05
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	pseudolongum	3.1	-2.2	0.5	-4.5	5.9e-06	2.5e-05
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	wittichii	4.2	-2.8	0.6	-4.5	6.1e-06	2.5e-05
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia		62.7	-3.3	0.7	-4.5	6.2e-06	2.6e-05
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus		44.6	-3.1	0.7	-4.5	8.5e-06	3.4e-05
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	johnsonii	4.2	-2.4	0.5	-4.5	8.5e-06	3.4e-05
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter		3.1	-2.3	0.5	-4.4	1.0e-05	3.9e-05
TM7	TM7-3	EW055				9.8	-2.7	0.6	-4.4	1.0e-05	3.9e-05
Actinobacteria	Actinobacteria	Actinomycetales	Beutenbergiaceae	Other	Other	5.7	-2.3	0.5	-4.4	1.1e-05	4.1e-05
Firmicutes	Bacilli	Bacillales	Planococcaceae	Planomicrobium		7.2	-2.4	0.5	-4.4	1.1e-05	4.4e-05
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Propionicimonas		3.5	-2.5	0.6	-4.3	1.5e-05	5.8e-05
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	coprophilus	3.7	-2.2	0.5	-4.3	1.6e-05	6.0e-05
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Brumimicrobium		3.0	-2.3	0.5	-4.3	1.9e-05	7.0e-05
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]		6.6	-2.4	0.6	-4.3	1.9e-05	7.1e-05

Firmicutes	Clostridia	Clostridiales	Clostridiaceae			111.2	-3.2	0.7	-4.3	1.9e-05	7.1e-05
Actinobacteria	Actinobacteria	Actinomycetales				2.9	-2.2	0.5	-4.2	2.2e-05	7.9e-05
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae			3.2	-2.2	0.5	-4.2	2.4e-05	8.7e-05
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Saccharopolyspora		3,6	-2.4	0.6	-4.2	2.4e-05	2.4e-05
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae			2.9	-2.2	0.5	-4.2	2.7e-05	9.7e-05
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter		4,9	-2.1	0.5	-4.2	2.9e-05	2.9e-05
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Aeromicrobium		3.2	-2.2	0.5	-4.2	2.9e-05	1.0e-04
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	equorum	6.7	-2.3	0.6	-4.2	3.1e-05	1.1e-04
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	prausnitzii	39.2	-3.1	0.8	-4.2	3.3e-05	1.1e-04
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae			24,8	-2.7	0.7	-4.1	4.4e-05	4.4e-05
Tenericutes	Mollicutes	RF39				3.0	-2.1	0.5	-4.1	5.0e-05	1.7e-04
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium		150.0	-3.1	0.8	-4.1	5.1e-05	1.7e-04
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			3.2	-2.0	0.5	-4.0	5.3e-05	1.8e-04
Proteobacteria	Deltaproteobacteria	GMD14H09				7.1	-2.3	0.6	-4.0	5.6e-05	1.8e-04
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae			5.3	-2.0	0.5	-4.0	6.5e-05	2.1e-04
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter		43.0	-2.8	0.7	-4.0	7.4e-05	2.4e-04
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae			4.2	2.8	0.7	4.0	7.7e-05	2.4e-04
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	Other	3.9	-1.9	0.5	-4.0	7.7e-05	2.4e-04
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Other	Other	56.1	-2.8	0.7	-3.9	7.9e-05	2.5e-04
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter		77.0	-3.3	0.8	-3.9	8.5e-05	2.7e-04
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	[Eubacterium]	biforme	3.9	-1.9	0.5	-3.9	9.2e-05	2.8e-04
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae			13.3	-2.4	0.6	-3.9	9.4e-05	2.9e-04
Actinobacteria	Actinobacteria	Actinomycetales	Other	Other	Other	2.8	-2.1	0.6	-3.9	1.1e-04	3.3e-04
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium		21.9	-2.4	0.6	-3.9	1.2e-04	3.5e-04
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter		3.2	-2.1	0.6	-3.9	1.2e-04	3.5e-04
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae			6.9	-2.2	0.6	-3.8	1.2e-04	3.5e-04
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Kineococcus		3.2	-2.2	0.6	-3.8	1.4e-04	4.2e-04
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia		3.1	-2.0	0.5	-3.8	1.5e-04	4.3e-04
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus		88.6	-2.9	0.8	-3.8	1.7e-04	4.9e-04

Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Candidatus Portiera		2,6	-2.0	0.5	-3.8	1.7e-04	1.7e-04
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Sedimentibacter		2.8	-2.1	0.6	-3.7	1.9e-04	5.5e-04
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella		88.7	-2.8	0.8	-3.7	2.1e-04	6.1e-04
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae			2.5	-1.8	0.5	-3.7	2.3e-04	6.4e-04
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Mogibacterium		2.4	-1.8	0.5	-3.7	2.3e-04	6.5e-04
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus		3.4	-1.9	0.5	-3.7	2.4e-04	6.7e-04
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae			3.5	-1.8	0.5	-3.7	2.5e-04	7.0e-04
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]		34.3	-2.7	0.7	-3.6	3.2e-04	8.9e-04
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	BD2-13		3.0	-1.9	0.5	-3.6	3.4e-04	9.4e-04
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	cryaerophilus	3.2	-2.0	0.6	-3.6	3.7e-04	1.0e-03
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter		3.0	-2.0	0.6	-3.5	3.9e-04	1.1e-03
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Aequorivita		2.3	-1.7	0.5	-3.5	4.1e-04	1.1e-03
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	RFN20		2.5	-1.8	0.5	-3.5	4.7e-04	1.3e-03
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola		2.3	-1.8	0.5	-3.5	4.8e-04	1.3e-03
Bacteroidetes	Bacteroidia	Bacteroidales	p-2534-18B5			2.5	-1.7	0.5	-3.5	5.2e-04	1.4e-03
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces		2.8	-1.8	0.5	-3.5	5.4e-04	1.4e-03
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Other	Other	6.8	-2.1	0.6	-3.4	5.6e-04	1.5e-03
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus	capsulatus	2.9	2.1	0.6	3.4	5.7e-04	1.5e-03
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Tissierella_Soehngenia		2,9	-1.8	0.5	-3.4	5.7e-04	5.7e-04
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Mycetocola		7.6	-2.0	0.6	-3.4	6.0e-04	1.5e-03
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Weeksella		2.6	-1.9	0.6	-3.4	6.3e-04	1.6e-03
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia		3.9	-2.0	0.6	-3.4	6.7e-04	1.7e-03
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae			2.5	-1.9	0.5	-3.4	7.3e-04	1.8e-03
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae			6.1	2.3	0.7	3.4	7.6e-04	1.9e-03
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium		2,5	-1.7	0.5	-3.4	7.8e-04	7.8e-04
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Anaerospora		2.4	-1.9	0.6	-3.3	8.2e-04	2.0e-03
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter		39.6	-2.7	0.8	-3.3	8.4e-04	2.1e-03
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium		7.2	2.5	0.7	3.3	9.0e-04	2.2e-03
[Thermi]	Deinococci	Deinococcales	Trueperaceae	B-42		2.5	-1.9	0.6	-3.3	9.5e-04	2.3e-03
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema		8.8	-2.1	0.6	-3.3	9.7e-04	2.3e-03

Firmicutes	Clostridia	Clostridiales	Peptococcaceae	rc4-4		2.1	-1.6	0.5	-3.3	1.1e-03	2.6e-03
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Marinobacter		2.3	-1.7	0.5	-3.3	1.1e-03	2.6e-03
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Proteiniclasticum		7,6	-1.9	0.6	-3.3	1.1e-03	1.1e-03
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Spirosoma		2.3	-1.8	0.6	-3.2	1.2e-03	2.7e-03
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia		8.5	-1.9	0.6	-3.2	1.2e-03	2.8e-03
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Other	2.4	-1.7	0.5	-3.2	1.2e-03	2.8e-03
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Clavibacter	Other	5.0	-1.9	0.6	-3.2	1.3e-03	3.0e-03
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri	55.4	-2.6	0.8	-3.2	1.4e-03	3.3e-03
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae			2.5	-1.5	0.5	-3.2	1.6e-03	3.6e-03
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Mannheimia		91.8	3.3	1.0	3.2	1.6e-03	3.6e-03
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium	acnes	6.0	-1.7	0.5	-3.2	1.6e-03	3.6e-03
Chloroflexi	Thermomicrobia	JG30-KF-CM45				2.3	-1.4	0.5	-3.1	1.6e-03	3.7e-03
Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae	Georgenia		2.1	-1.5	0.5	-3.1	1.8e-03	4.0e-03
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Mycoplasma		35956.8	2.3	0.7	3.1	1.8e-03	4.0e-03
Actinobacteria	Actinobacteria	Actinomycetales	Yaniellaceae	Yaniella		4.5	-1.6	0.5	-3.0	2.4e-03	5.2e-03
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Gelidibacter		2.2	-1.6	0.5	-3.0	2.4e-03	5.2e-03
Firmicutes	Bacilli	Bacillales	Planococcaceae			2.1	-1.5	0.5	-3.0	2.4e-03	5.2e-03
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Other	Other	3.9	-1.6	0.5	-3.0	2.6e-03	5.7e-03
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Prauserella	rugosa	2.4	-1.7	0.6	-3.0	2.6e-03	5.7e-03
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	Other	2.0	-1.5	0.5	-3.0	2.6e-03	5.7e-03
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]			6.4	-1.7	0.6	-3.0	2.9e-03	6.1e-03
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus		2.0	-1.5	0.5	-3.0	3.0e-03	6.3e-03
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas		3.9	1.9	0.6	2.9	3.2e-03	6.8e-03
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia		2.6	-1.5	0.5	-2.9	3.9e-03	8.1e-03
Proteobacteria	Alphaproteobacteria	Sphingomonadales				2.8	-1.5	0.5	-2.9	4.2e-03	8.9e-03
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae			3.1	-1.4	0.5	-2.9	4.3e-03	8.9e-03
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia		2.3	-1.3	0.5	-2.8	4.4e-03	9.1e-03
Actinobacteria	Actinobacteria	Actinomycetales	Sanguibacteraceae	Sanguibacter		5.6	-1.8	0.6	-2.8	4.6e-03	9.5e-03
Proteobacteria	Gammaproteobacteria	Alteromonadales				2.1	-1.3	0.5	-2.8	4.8e-03	9.8e-03

Firmicutes	Bacilli	Bacillales	Bacillaceae	Natronobacillus		2.3	-1.5	0.5	-2.8	5.0e-03	1.0e-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	eutactus	2.2	-1.6	0.6	-2.8	5.1e-03	1.0e-02
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	variabile	2.3	-1.4	0.5	-2.8	5.3e-03	1.1e-02
Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacterineae			2.1	-1.5	0.5	-2.8	5.7e-03	1.1e-02
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Dyadobacter		4.5	-1.6	0.6	-2.8	5.8e-03	1.2e-02
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma		13.4	-1.9	0.7	-2.7	6.5e-03	1.3e-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	Other	2.0	-1.4	0.5	-2.7	6.6e-03	1.3e-02
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]			13.1	-1.9	0.7	-2.7	6.6e-03	1.3e-02
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	p-1630-c5	1.9	-1.5	0.5	-2.7	6.8e-03	1.3e-02
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Gallicola		1.9	-1.3	0.5	-2.7	6.9e-03	1.3e-02
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	faecium	1.9	-1.3	0.5	-2.7	7.0e-03	1.4e-02
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Rathayibacter	caricis	5.5	-1.6	0.6	-2.6	8.6e-03	1.7e-02
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Cloacibacterium		2.1	1.4	0.6	2.6	9.2e-03	1.8e-02
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Ornithobacterium		2,9	-1.4	0.6	-2.6	9.4e-03	9.4e-03
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae			3.8	-1.5	0.6	-2.6	1.0e-02	1.9e-02
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio		1.8	-1.3	0.5	-2.6	1.0e-02	1.9e-02
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium		9.7	-1.7	0.7	-2.6	1.0e-02	1.9e-02
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	flavefaciens	1.8	-1.3	0.5	-2.6	1.0e-02	1.9e-02
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas		4.7	-1.5	0.6	-2.5	1.1e-02	2.0e-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus		33.4	-2.0	0.8	-2.5	1.1e-02	2.0e-02
Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	metschnikovii	2.0	-1.4	0.6	-2.5	1.1e-02	2.0e-02
Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae			4.0	-1.4	0.6	-2.5	1.1e-02	2.0e-02
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	cryoconitis	2.3	-1.2	0.5	-2.5	1.1e-02	2.1e-02
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas		2.2	-1.2	0.5	-2.5	1.2e-02	2.2e-02
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	eggerthii	1.9	1.5	0.6	2.5	1.3e-02	2.4e-02
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella		1.9	1.5	0.6	2.4	1.5e-02	2.8e-02
Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina		1.8	-1.2	0.5	-2.4	1.6e-02	2.8e-02
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	[Eubacterium]	dolichum	1.7	-1.2	0.5	-2.4	1.6e-02	2.8e-02
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	diminuta	1.7	1.4	0.6	2.4	1.7e-02	3.0e-02

Firmicutes	Clostridia	Clostridiales	Christensenellaceae			1.8	-1.2	0.5	-2.4	1.8e-02	3.2e-02
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Trueperella		2.1	-1.2	0.5	-2.4	1.8e-02	3.3e-02
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus		3.9	1.4	0.6	2.4	1.8e-02	3.3e-02
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae			4.2	-1.3	0.5	-2.4	1.9e-02	3.3e-02
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium		10,4	-1.5	0.6	-2.3	1.9e-02	1.9e-02
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus		3.0	-1.1	0.5	-2.3	2.0e-02	3.4e-02
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira		1.9	-1.2	0.5	-2.3	2.0e-02	3.5e-02
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Other	9.6	-1.6	0.7	-2.3	2.0e-02	3.5e-02
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas		1.8	1.4	0.6	2.3	2.1e-02	3.5e-02
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Sharpea	p-3329-23G2	2.0	-1.3	0.6	-2.3	2.2e-02	3.8e-02
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae			5.6	-1.3	0.6	-2.3	2.4e-02	4.0e-02
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Other	2.8	-1.1	0.5	-2.2	2.5e-02	4.3e-02
Firmicutes	Bacilli	Lactobacillales				1.8	-1.1	0.5	-2.2	2.7e-02	4.6e-02
Proteobacteria	Alphaproteobacteria	RF32				3.9	-1.3	0.6	-2.2	2.8e-02	4.7e-02
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas		7.7	-1.4	0.6	-2.2	2.9e-02	4.8e-02
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Kocuria	rhizophila	2.2	-1.1	0.5	-2.1	0.0	0.1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Other	Other	1.5	1.2	0.6	2.1	0.0	0.1
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	longum	1.8	-1.2	0.5	-2.1	0.0	0.1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae			1.8	-1.1	0.5	-2.1	0.0	0.1
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium		2.3	-1.0	0.5	-2.1	0.0	0.1
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus		1.6	1.2	0.6	2.1	0.0	0.1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	echinoides	3.4	-1.1	0.5	-2.0	0.0	0.1
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera		1.6	-1.0	0.5	-2.0	0.0	0.1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium		41.0	-1.7	0.8	-2.0	0.0	0.1
Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Cardiobacterium		1.5	1.2	0.6	2.0	0.0	0.1
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Parvimonas		1.5	1.1	0.6	2.0	0.0	0.1
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Pigmentiphaga		1.7	-1.0	0.5	-2.0	0.0	0.1
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Nesterenkonia		1.7	-1.0	0.5	-2.0	0.0	0.1
Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	Methylophaga		1.6	-1.0	0.5	-2.0	0.0	0.1
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae			1.7	-1.0	0.5	-2.0	0.0	0.1

Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium		27,4	-1.5	0.8	-1.9	5.2e-02	0.1
Firmicutes	Bacilli	Lactobacillales	Other	Other	Other	1.7	-1.0	0.5	-1.9	0.1	0.1
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus		1,9	1.2	0.6	1.9	5.2e-02	0.1
Bacteroidetes	Bacteroidia	Bacteroidales	[Odoribacteraceae]	Odoribacter		1.7	-1.0	0.5	-1.9	0.1	0.1
[Thermi]	Deinococci	Thermales	Thermaceae	Meiothermus		1.5	1.1	0.6	1.9	0.1	0.1
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae			1.9	1.0	0.5	1.9	0.1	0.1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	Other	2.6	-1.0	0.5	-1.9	0.1	0.1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	yabuuchiae	2.7	1.1	0.6	1.9	0.1	0.1
Cyanobacteria	Oscillatoriophycideae	Chroococcales	Xenococcaceae	Chroococcidiopsis		1,4	1.1	0.6	1.8	6.5e-02	0.1
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae			1.7	-1.0	0.6	-1.8	0.1	0.1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Other	4.1	-1.1	0.6	-1.8	0.1	0.1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leucobacter		2.7	-0.9	0.5	-1.8	0.1	0.1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium		1,5	1.0	0.5	1.8	6.9e-02	0.1
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus		1.8	-1.0	0.6	-1.8	0.1	0.1
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Other	Other	1.6	-0.9	0.5	-1.8	0.1	0.1
Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae			1.4	1.0	0.6	1.8	0.1	0.1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	Other	1.4	1.0	0.6	1.8	0.1	0.1
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae			3.9	-1.0	0.6	-1.7	0.1	0.1
Other	Other	Other	Other	Other	Other	39.9	-1.2	0.7	-1.7	0.1	0.1
Actinobacteria	Acidimicrobiia	Acidimicrobiales				1.7	-0.8	0.5	-1.7	0.1	0.1
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Peptoniphilus		1.7	1.0	0.6	1.7	0.1	0.1
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium		1.7	-0.9	0.5	-1.7	0.1	0.1
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	Other	Other	2.8	-1.0	0.6	-1.7	0.1	0.1
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Micrococcus		2.4	-0.8	0.5	-1.7	0.1	0.1
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides		1.5	-0.8	0.5	-1.7	0.1	0.1
Proteobacteria	Gammaproteobacteria	Alteromonadales	[Chromatiaceae]			1.6	-0.8	0.5	-1.7	0.1	0.1
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae			2.7	-0.9	0.5	-1.6	0.1	0.1
Proteobacteria	Gammaproteobacteria	Alteromonadales	[Chromatiaceae]	Rheinheimera		1.7	-0.9	0.6	-1.6	0.1	0.2
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	Other	1.6	-0.8	0.5	-1.6	0.1	0.2

Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae			1.6	-0.9	0.5	-1.6	0.1	0.2
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Desemzia		1.5	-0.8	0.5	-1.6	0.1	0.2
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium		2.8	-0.8	0.5	-1.6	0.1	0.2
Bacteroidetes	Bacteroidia	Bacteroidales	[Barnesiellaceae]			1.6	-0.8	0.5	-1.5	0.1	0.2
Bacteroidetes	[Saprospirae]	[Saprospirales]				1.6	-0.8	0.5	-1.5	0.1	0.2
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Other	Other	1.6	-0.8	0.6	-1.5	0.1	0.2
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter		1.8	-0.7	0.5	-1.5	0.1	0.2
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	minor	1.6	-0.8	0.5	-1.5	0.1	0.2
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera		1.6	-0.8	0.5	-1.5	0.1	0.2
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	plebeius	1.6	-0.7	0.5	-1.5	0.1	0.2
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	marcusii	2.9	-0.8	0.6	-1.4	0.1	0.2
Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae	Xylanimicrobium	Other	1.5	-0.7	0.5	-1.4	0.2	0.2
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Marinilactibacillus	psychrotolerans	1.6	-0.8	0.6	-1.4	0.2	0.2
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Other	1.7	-0.7	0.5	-1.4	0.2	0.2
Firmicutes	Bacilli	Bacillales	Planococcaceae	Other	Other	1.5	-0.7	0.5	-1.4	0.2	0.2
Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Pseudidiomarina		1.6	-0.7	0.5	-1.4	0.2	0.2
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas		1.8	0.7	0.5	1.4	0.2	0.2
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	Deinococcus		2.4	-0.7	0.5	-1.4	0.2	0.2
Cyanobacteria	Chloroplast	Chlorophyta				1.6	-0.7	0.5	-1.4	0.2	0.2
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	multivorum	1.6	-0.7	0.5	-1.4	0.2	0.2
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]			1.6	-0.8	0.6	-1.4	0.2	0.2
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Kineococcus	Other	1.9	-0.7	0.5	-1.3	0.2	0.2
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	gen-68		1.6	-0.8	0.6	-1.3	0.2	0.2
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia		3.5	0.8	0.6	1.3	0.2	0.2
Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia	Other	1.4	-0.6	0.5	-1.3	0.2	0.3
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae			1.5	-0.6	0.5	-1.3	0.2	0.3
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Salinicoccus		2.0	-0.7	0.5	-1.3	0.2	0.3
Proteobacteria	Alphaproteobacteria	Rhizobiales				2.9	-0.7	0.5	-1.3	0.2	0.3

Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae			3.3	0.8	0.6	1.3	0.2	0.3
Actinobacteria	Actinobacteria	Actinomycetales	Jonesiaceae	Jonesia		1.5	-0.7	0.5	-1.3	0.2	0.3
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Anaerococcus		1.6	-0.6	0.5	-1.3	0.2	0.3
Planctomycetes	Phycisphaerae	WD2101				1.5	-0.7	0.5	-1.2	0.2	0.3
SR1						1.4	-0.6	0.5	-1.2	0.2	0.3
Firmicutes	Clostridia	OPB54				1.5	-0.7	0.6	-1.2	0.2	0.3
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae			7.0	-0.6	0.5	-1.2	0.2	0.3
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae			6.5	1.0	0.8	1.2	0.2	0.3
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Helcococcus		3.0	-0.7	0.5	-1.2	0.2	0.3
Tenericutes	RF3	ML615J-28				1.4	-0.6	0.5	-1.2	0.2	0.3
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	[Eubacterium]	cylindroides	1.4	-0.6	0.5	-1.2	0.2	0.3
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Myroides		2.4	-0.7	0.6	-1.2	0.2	0.3
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	mizutaii	1.4	-0.6	0.5	-1.2	0.2	0.3
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	Other	1.9	0.6	0.5	1.2	0.2	0.3
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	brevis	1.5	-0.6	0.5	-1.2	0.2	0.3
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella		26.1	-0.8	0.7	-1.1	0.3	0.3
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	Other	1.5	-0.6	0.5	-1.1	0.3	0.3
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Other	1.4	-0.6	0.5	-1.1	0.3	0.3
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium		78.3	-0.8	0.7	-1.1	0.3	0.3
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Other	Other	1.5	-0.6	0.5	-1.1	0.3	0.3
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter		1.5	-0.6	0.5	-1.1	0.3	0.3
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter		1.5	-0.6	0.5	-1.1	0.3	0.3
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Other	Other	1.5	-0.6	0.6	-1.1	0.3	0.3
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc		1.6	-0.6	0.5	-1.1	0.3	0.3
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Dysgonomonas		1.4	-0.6	0.5	-1.1	0.3	0.3
Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae	Xylanimicrobium		1.4	-0.6	0.5	-1.1	0.3	0.3
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum		2.4	-0.6	0.6	-1.1	0.3	0.3
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]	Other	1.4	-0.5	0.5	-1.1	0.3	0.3
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Adlercreutzia		1.5	-0.5	0.5	-1.1	0.3	0.3

Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Alkalibacter		1.5	-0.6	0.5	-1.0	0.3	0.4
Chloroflexi	Ellin6529					1.5	-0.5	0.5	-1.0	0.3	0.4
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae			1.5	-0.5	0.5	-1.0	0.3	0.4
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae			1.5	-0.5	0.5	-1.0	0.3	0.4
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dokdonella		1.5	-0.6	0.6	-1.0	0.3	0.4
TM7	TM7-1					2.1	-0.5	0.5	-1.0	0.3	0.4
TM7	TM7-3	Blgi18				1.5	-0.6	0.6	-1.0	0.3	0.4
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Peptococcus		1.5	-0.5	0.5	-1.0	0.3	0.4
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	1.9	-0.6	0.6	-1.0	0.3	0.4
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Succiniclasticum		2.0	0.5	0.5	0.9	0.3	0.4
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Other	Other	2.0	-0.5	0.5	-0.9	0.4	0.4
Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Other	Other	1.5	-0.5	0.5	-0.9	0.4	0.4
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium		1.6	-0.4	0.5	-0.9	0.4	0.4
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	ph2		1.5	-0.5	0.5	-0.9	0.4	0.4
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	stutzeri	2.2	-0.5	0.6	-0.9	0.4	0.4
Actinobacteria	Actinobacteria	Actinomycetales	Williamsiaceae	Williamsia		1.4	-0.5	0.5	-0.9	0.4	0.4
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Acetobacterium		1.4	-0.5	0.6	-0.9	0.4	0.4
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Other	Other	Other	1.4	-0.4	0.5	-0.9	0.4	0.4
Spirochaetes	Spirochaetes	Sphaerochaetales	Sphaerochaetaceae	Sphaerochaeta		1.4	-0.5	0.5	-0.9	0.4	0.4
TM7	TM7-3	I025				1.4	-0.4	0.5	-0.8	0.4	0.5
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Demequina		1.5	-0.4	0.5	-0.8	0.4	0.5
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus		2.2	-0.4	0.5	-0.8	0.4	0.5
Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111			1.5	-0.4	0.5	-0.8	0.4	0.5
Proteobacteria	Alphaproteobacteria					1.6	-0.4	0.5	-0.8	0.4	0.5
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolinaceae	Anaerolinea		1.4	-0.4	0.5	-0.7	0.5	0.5
Firmicutes	Bacilli	Haloplasmatales	Haloplasmataceae			1.4	-0.4	0.5	-0.7	0.5	0.5
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	p-75-a5		1.5	-0.4	0.5	-0.7	0.5	0.5
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Thauera		1.5	-0.4	0.5	-0.7	0.5	0.5
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae			1088.7	-0.7	0.9	-0.7	0.5	0.5
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	succinogenes	3.4	-0.4	0.6	-0.7	0.5	0.5

Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	neonatale	2.5	-0.4	0.5	-0.7	0.5	0.5
Verrucomicrobia	Verruco-5					1.4	-0.4	0.5	-0.7	0.5	0.5
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Cryocola		1.4	-0.3	0.5	-0.7	0.5	0.6
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	Other	1.4	-0.3	0.5	-0.6	0.5	0.6
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacteriu m	pilosum	1.4	-0.3	0.5	-0.6	0.5	0.6
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella		1.4	-0.3	0.5	-0.6	0.6	0.6
Actinobacteria	Actinobacteria	Actinomycetales	Gordoniaceae	Gordonia		1.4	-0.3	0.5	-0.6	0.6	0.6
Firmicutes	Bacilli	Bacillales	Bacillaceae			11.0	-0.3	0.5	-0.6	0.6	0.6
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Pseudoramibacter_Eubacterium		1,4	-0.3	0.5	-0.6	5.7e-01	0.6
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agrococcus		1.4	-0.3	0.5	-0.6	0.6	0.6
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	cereus	1.7	0.3	0.5	0.5	0.6	0.6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	leguminosarum	1.7	0.2	0.5	0.5	0.6	0.6
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Other	Other	808.3	-0.4	0.8	-0.5	0.6	0.7
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae			2.5	0.2	0.5	0.4	0.7	0.7
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]			5.3	-0.2	0.6	-0.4	0.7	0.7
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	bromii	1.5	0.2	0.5	0.4	0.7	0.8
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Tindallia_Anoxynatronum		1,5	-0.2	0.5	-0.3	7.4e-01	0.7
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	1.4	0.2	0.5	0.3	0.8	0.8
Bacteroidetes	Sphingobacteriia	Sphingobacteriales				1.4	0.2	0.5	0.3	0.8	0.8
Firmicutes	Bacilli	Bacillales	Bacillaceae	Other	Other	1.5	-0.1	0.5	-0.3	0.8	0.8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae			1.6	-0.1	0.5	-0.2	0.8	0.8
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Delftia		38.8	0.1	0.6	0.1	0.9	0.9
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae			1.8	-0.1	0.5	-0.1	0.9	0.9
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	YRC22		1.4	-0.1	0.5	-0.1	0.9	0.9
Bacteroidetes	Cytophagia	Cytophagales	Cyclobacteriaceae			1.8	0.0	0.5	-0.1	0.9	0.9
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium		1,5	0.0	0.5	-0.1	9.5e-01	0.9
Cyanobacteria	Chloroplast	Stramenopiles				1.6	0.0	0.5	0.0	1.0	1.0
Proteobacteria	Gammaproteobacteria	Alteromonadales	211ds20			1.8	0.0	0.5	0.0	1.0	1.0
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Other	Other	2.0	0.0	0.5	0.0	1.0	1.0

Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes		1.4	0.0	0.5	0.0	1.0	1.0
Euryarchaeota	Thermoplasmata	E2	[Methanomassiliicoccaceae]	vadinCA11		1.3	0.9	0.6	1.6	0.1	NA
Acidobacteria	Acidobacteria-6	CCU21				1.2	0.3	0.6	0.5	0.6	NA
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae			1.1	0.3	0.5	0.5	0.6	NA
Acidobacteria	[Chloracidobacteria]	RB41	Ellin6075			1.2	0.2	0.6	0.3	0.7	NA
Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017			1.3	-0.1	0.5	-0.2	0.8	NA
Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1			1.2	0.2	0.6	0.3	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Other	Other	1.2	0.2	0.6	0.3	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces		1.3	0.4	0.5	0.7	0.5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Varibaculum		1.2	0.7	0.6	1.2	0.2	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinopolysporaceae			1.3	0.1	0.5	0.2	0.8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Beutenbergiaceae	Salana	multivorans	1.3	-0.4	0.5	-0.8	0.4	NA
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Other	Other	1.3	0.1	0.5	0.2	0.8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Other	Other	1.2	0.1	0.5	0.2	0.9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium	Other	1.1	0.4	0.5	0.8	0.4	NA
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium		1.3	-0.1	0.5	-0.2	0.9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Dermabacter		1.2	0.1	0.5	0.2	0.8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae			1.2	0.3	0.6	0.5	0.6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Arsenicicoccus		1.3	0.0	0.5	-0.1	0.9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Phycicoccus		1.2	0.2	0.5	0.3	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Terracoccus		1.3	-0.5	0.5	-1.0	0.3	NA
Actinobacteria	Actinobacteria	Actinomycetales	Jonesiaceae			1.2	-0.4	0.5	-0.7	0.5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Other	Other	1.3	0.5	0.5	1.0	0.3	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agrococcus	Other	1.2	0.2	0.5	0.3	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agrococcus	jenensis	1.2	0.3	0.5	0.6	0.5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Curtobacterium		1.2	0.1	0.5	0.1	0.9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Other	Other	1.2	0.4	0.5	0.8	0.4	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Microbispora	rosea	1.2	0.3	0.5	0.5	0.6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	aeria	1.2	0.3	0.6	0.5	0.6	NA

Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	dentocariosa	1.3	-0.2	0.5	-0.4	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae			1.1	0.5	0.6	0.8	0.4	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae			1.3	0.5	0.5	0.9	0.4	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae			1.2	0.1	0.5	0.1	0.9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Friedmanniella		1.2	0.2	0.5	0.3	0.8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	plantarum	1.2	-0.2	0.5	-0.3	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiopsaceae	Other	Other	1.2	0.3	0.5	0.5	0.6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiopsaceae			1.1	0.5	0.6	0.8	0.4	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiopsaceae	Nocardiopsis	exhalans	1.2	0.2	0.5	0.3	0.7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae	Other	Other	1.2	0.1	0.5	0.1	0.9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Luteococcus		1.3	0.0	0.5	0.0	1.0	NA
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia		1.1	0.4	0.5	0.7	0.5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Saccharomonospora		1,1	0.3	0.5	0.6	5.5e-01	0.5
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Saccharopolyspora	hirsuta	1.3	-0.1	0.5	-0.2	0.8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Ruaniaceae	Other	Other	1.3	-0.3	0.5	-0.5	0.6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Sanguibacteraceae	Sanguibacter	Other	1.2	0.3	0.6	0.6	0.6	NA
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella		1.1	0.5	0.6	0.8	0.4	NA
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella	aerofaciens	1.2	0.1	0.5	0.1	0.9	NA
Actinobacteria	Nitriliruptoria	Euzebyales	Euzebyaceae	Euzebya		1.1	0.3	0.5	0.6	0.5	NA
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae			1.2	0.1	0.5	0.1	0.9	NA
Actinobacteria	Thermoleophilia	Solirubrobacterales				1.3	0.0	0.5	-0.1	0.9	NA
Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	Patulibacter		1.2	0.1	0.5	0.3	0.8	NA
Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae			1.2	-0.5	0.5	-0.9	0.4	NA
Armatimonadetes	SHA-37					1.2	0.2	0.6	0.3	0.7	NA
Armatimonadetes	SJA-176	TP122				1.2	0.3	0.6	0.5	0.6	NA
BRC1	PRR-11					1.2	0.3	0.6	0.5	0.6	NA
Bacteroidetes	Bacteroidia	Bacteroidales	BS11			1.1	0.5	0.6	0.8	0.4	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	BF311		1.1	0.2	0.5	0.3	0.8	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Other	1.4	0.0	0.5	-0.1	1.0	NA

Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	barnesiae	1.2	0.7	0.6	1.2	0.2	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	fragilis	1.1	0.2	0.5	0.4	0.7	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	uniformis	1.2	0.1	0.5	0.1	0.9	NA
Bacteroidetes	Bacteroidia	Bacteroidales	GZKB119			1.2	0.2	0.6	0.3	0.7	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	endodontalis	1.3	0.8	0.6	1.5	0.1	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	intermedia	1.2	0.8	0.6	1.3	0.2	NA
Bacteroidetes	Bacteroidia	Bacteroidales	SB-1			1.2	0.2	0.6	0.3	0.7	NA
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]			1.2	-0.2	0.5	-0.4	0.7	NA
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	Paraprevotella		1.1	0.4	0.5	0.8	0.4	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae			1.3	0.0	0.5	-0.1	0.9	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga		1.3	-0.1	0.5	-0.3	0.8	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flectobacillus		1.3	-0.1	0.5	-0.3	0.8	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Leadbetterella		1.2	-0.2	0.5	-0.4	0.7	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Rudanella		1.2	0.4	0.5	0.8	0.4	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Cryomorpha		1.2	0.2	0.6	0.3	0.7	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga		1.2	0.7	0.6	1.2	0.2	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	succinicans	1.3	-0.2	0.5	-0.4	0.7	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Zhouia		1.4	-0.3	0.5	-0.6	0.6	NA
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Other	Other	1.3	-0.3	0.5	-0.6	0.5	NA
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Olivibacter		1.3	-0.3	0.5	-0.6	0.5	NA
Bacteroidetes	[Rhodothermi]	[Rhodothermales]	[Balneolaceae]			1.2	0.3	0.6	0.5	0.6	NA
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Chitinophaga		1.3	-0.1	0.5	-0.3	0.8	NA
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Flavisolibacter		1.3	0.0	0.5	0.0	1.0	NA
Chlamydiae	Chlamydiia	Chlamydiales	Chlamydiaceae	Chlamydia	Other	1.1	0.3	0.6	0.6	0.5	NA
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae			1.1	0.2	0.5	0.4	0.7	NA
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	Caldilinea		1.3	-0.1	0.5	-0.3	0.8	NA
Chloroflexi	Chloroflexi	[Roseiflexales]				1.2	0.1	0.5	0.1	0.9	NA
Cyanobacteria	ML635J-21					1.2	-0.2	0.5	-0.5	0.6	NA
Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae			1.2	0.3	0.6	0.5	0.6	NA

Fibrobacteres	Fibrobacteria	258ds10				1.3	-0.2	0.5	-0.4	0.7	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Other	1.3	0.7	0.5	1.3	0.2	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	flexus	1.2	0.8	0.6	1.3	0.2	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	thermoamylovorans	1.3	-0.1	0.5	-0.3	0.8	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Marinococcus		1.3	0.0	0.5	-0.1	1.0	NA
Firmicutes	Bacilli	Bacillales	Paenibacillaceae			1.1	0.4	0.5	0.7	0.5	NA
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	boronitolerans	1.4	-0.4	0.5	-0.8	0.4	NA
Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae			1,3	0.0	0.5	-0.1	9.5E-01	1.0
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Alkalibacterium	Other	1.2	0.1	0.5	0.1	0.9	NA
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Alkalibacterium		1.4	-0.3	0.5	-0.5	0.6	NA
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium		1.3	-0.3	0.5	-0.7	0.5	NA
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	Other	1.2	0.2	0.5	0.3	0.7	NA
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	cecorum	1.2	0.2	0.6	0.3	0.7	NA
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	agilis	1.2	-0.1	0.5	-0.1	0.9	NA
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	reuteri	1.2	0.3	0.6	0.5	0.6	NA
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	ruminis	1.2	-0.2	0.5	-0.4	0.7	NA
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	Other	1.2	0.1	0.5	0.1	0.9	NA
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus		1.3	-0.1	0.5	-0.2	0.8	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Other	Other	1.3	0.0	0.5	0.0	1.0	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Candidatus Arthromitus		1,3	-0.1	0.5	-0.3	8.0e-01	0.8
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	hiranonis	1.1	0.5	0.6	0.8	0.4	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	perfringens	1.2	-0.1	0.5	-0.1	0.9	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	SMB53		1.2	0.1	0.5	0.1	0.9	NA
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae			1.1	0.3	0.5	0.5	0.6	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium		1.4	-0.5	0.5	-1.1	0.3	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	faecis	1.2	0.2	0.5	0.3	0.8	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]	gnavus	1.2	0.3	0.6	0.6	0.6	NA
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Niigata-25		1.4	-0.2	0.5	-0.4	0.7	NA
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Filifactor		1.3	0.9	0.6	1.6	0.1	NA

Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus	Other	1.2	0.7	0.6	1.2	0.2	NA
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Other	Other	1.2	0.8	0.6	1.3	0.2	NA
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerofilum		1.1	0.4	0.5	0.7	0.5	NA
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	Other	1.2	0.3	0.6	0.5	0.6	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae			1.2	0.6	0.5	1.1	0.3	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Acidaminococcus		1.2	0.3	0.6	0.5	0.6	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Dialister		1.1	0.4	0.5	0.8	0.4	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megamonas		1.2	0.1	0.5	0.1	0.9	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Mitsuokella		1.2	0.3	0.6	0.5	0.6	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	ruminantium	1.1	0.3	0.5	0.5	0.6	NA
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Other	Other	1.2	0.2	0.5	0.3	0.7	NA
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Finegoldia		1.1	0.6	0.6	1.0	0.3	NA
Firmicutes	Clostridia	MBA08				1.2	-0.2	0.5	-0.5	0.6	NA
Firmicutes	Clostridia	Natranaerobiales				1.3	0.2	0.5	0.4	0.7	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Catenibacterium		1.2	0.2	0.6	0.3	0.7	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Sharpea	Other	1.2	0.2	0.5	0.4	0.7	NA
GN02	BD1-5					1.2	0.3	0.6	0.5	0.6	NA
Lentisphaerae	[Lentisphaeria]	Victivallales	Victivallaceae			1.3	0.4	0.5	0.8	0.4	NA
Lentisphaerae	[Lentisphaeria]	Z20	R4-45B			1.2	0.1	0.5	0.1	0.9	NA
OD1						1.2	0.6	0.6	1.0	0.3	NA
Planctomycetes	Phycisphaerae	Phycisphaerales				1.2	0.5	0.6	0.9	0.4	NA
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae			1.3	0.3	0.5	0.6	0.5	NA
Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae			1.2	0.1	0.5	0.1	0.9	NA
Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces		1.2	0.3	0.6	0.6	0.6	NA
Proteobacteria	Alphaproteobacteria	BD7-3				1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Other	Other	1.2	0.1	0.5	0.1	0.9	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bartonellaceae			1.3	0.0	0.5	-0.1	0.9	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae			1.3	0.9	0.6	1.6	0.1	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Other	Other	1.3	-0.3	0.5	-0.7	0.5	NA

Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	intermedium	1.2	0.3	0.6	0.6	0.6	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	Other	1.1	0.4	0.5	0.8	0.4	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1.2	0.5	0.6	0.9	0.4	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium		1.2	0.7	0.6	1.2	0.2	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Nitratireductor		1.2	0.2	0.6	0.3	0.7	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Phyllobacterium		1.2	-0.1	0.5	-0.2	0.9	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Other	Other	1.1	0.4	0.5	0.7	0.5	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Labrys		1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter		1.1	0.6	0.6	1.0	0.3	NA
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Other	Other	1.3	-0.1	0.5	-0.2	0.8	NA
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acetobacter		1.3	-0.1	0.5	-0.3	0.8	NA
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella		1.2	0.1	0.5	0.1	0.9	NA
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Other	Other	1.3	-0.2	0.5	-0.4	0.7	NA
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	capsulatum	1.2	0.8	0.6	1.3	0.2	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter		1.2	0.7	0.6	1.2	0.2	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Lautropia		1.3	0.1	0.5	0.2	0.8	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax		1.2	-0.3	0.5	-0.5	0.6	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hydrogenophaga		1.2	-0.2	0.5	-0.3	0.7	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hylemonella		1.2	0.2	0.6	0.3	0.7	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Methylibium		1.2	0.1	0.5	0.1	0.9	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Tepidimonas		1.2	0.7	0.6	1.2	0.2	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax		1.1	0.5	0.6	0.8	0.4	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Other	Other	1.3	-0.1	0.5	-0.2	0.9	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Cupriavidus	Other	1.2	0.3	0.5	0.5	0.6	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	lividum	1.1	0.4	0.5	0.8	0.4	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Ralstonia		1.3	0.2	0.5	0.4	0.7	NA

Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylotenera	mobilis	1.2	0.1	0.5	0.1	0.9	NA
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Vitreoscilla		1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Vogesella		1.4	-0.3	0.5	-0.6	0.6	NA
Proteobacteria	Betaproteobacteria	Procabacteriales	Procabacteriaceae	Other	Other	1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae			1.3	-0.5	0.5	-1.0	0.3	NA
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Propionivibrio		1.2	0.1	0.5	0.2	0.8	NA
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea		1.2	0.2	0.6	0.3	0.7	NA
Proteobacteria	Deltaproteobacteria					1.1	0.2	0.5	0.3	0.8	NA
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae			1.3	-0.2	0.5	-0.5	0.7	NA
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus	Other	1.2	0.2	0.5	0.3	0.7	NA
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus		1.3	-0.4	0.5	-0.9	0.4	NA
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio		1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Deltaproteobacteria	FAC87				1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Deltaproteobacteria	Myxococcales				1.4	-0.6	0.5	-1.3	0.2	NA
Proteobacteria	Deltaproteobacteria	Myxococcales	0319-6G20			1.1	0.5	0.6	0.8	0.4	NA
Proteobacteria	Deltaproteobacteria	Spirobacillales				1.2	0.5	0.5	0.9	0.4	NA
Proteobacteria	Gammaproteobacteria	Other	Other	Other	Other	1.3	-0.4	0.5	-0.8	0.4	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Other	Other	1.3	0.5	0.5	0.9	0.4	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	ND137		1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	HTCC2188	HTCC		1.2	-0.1	0.5	-0.2	0.9	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	[Chromatiaceae]	Rheinheimera	Other	1.3	0.0	0.5	0.0	1.0	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Other	Other	1.3	-0.3	0.5	-0.7	0.5	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Other	1.2	0.1	0.5	0.1	0.9	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella		1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Morganella	morganii	1.3	0.6	0.6	1.1	0.3	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	agglomerans	1.1	0.4	0.5	0.8	0.4	NA
Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae			1.1	0.2	0.5	0.4	0.7	NA
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae			1.4	-0.3	0.5	-0.6	0.6	NA
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	Other	1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	Alcanivorax		1.4	-0.4	0.5	-0.8	0.4	NA

Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae			1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Oleibacter		1.4	-0.3	0.5	-0.6	0.6	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Bibersteinia	Other	1.3	0.2	0.5	0.4	0.7	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Chelonobacter	Taxon	1.1	0.4	0.5	0.8	0.4	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae	1.1	0.4	0.6	0.8	0.4	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella	Other	1.3	0.8	0.6	1.5	0.1	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella		1.2	0.7	0.6	1.3	0.2	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Other	Other	Other	1.2	0.5	0.6	0.9	0.4	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Other	1.2	-0.2	0.5	-0.3	0.7	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	schindleri	1.3	0.4	0.5	0.7	0.5	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Alkanindiges		1.2	0.3	0.6	0.5	0.6	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	pacificensis	1.3	-0.6	0.5	-1.1	0.3	NA
Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae			1,2	0.3	0.6	0.5	5.9e-01	0.6
Proteobacteria	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	Pseudoalteromonas		1,2	0.3	0.6	0.6	5.8e-01	0.6
Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio		1.4	-0.3	0.5	-0.6	0.6	NA
Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	rumoiensis	1.2	-0.2	0.5	-0.5	0.6	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Nevskia		1.2	0.4	0.5	0.8	0.4	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Other	Other	1.2	0.1	0.5	0.1	0.9	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteibacter	rhizovicinus	1.3	-0.7	0.5	-1.4	0.2	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Rhodanobacter		1.2	0.7	0.6	1.2	0.2	NA
Spirochaetes	Spirochaetes	M2PT2-76				1.2	0.2	0.6	0.3	0.7	NA
Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	Pyramidobacter		1.2	-0.4	0.5	-0.8	0.4	NA
TM6	SJA-4					1.2	0.3	0.5	0.7	0.5	NA
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Mycoplasma	Other	1.1	0.5	0.6	0.8	0.4	NA
Verrucomicrobia	Opitutae	Puniceicoccales	Puniceicoccaceae			1.2	-0.1	0.5	-0.1	0.9	NA
Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]			1.1	0.5	0.6	0.8	0.4	NA
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	muciniphila	1.3	-0.1	0.5	-0.3	0.8	NA
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter		1.4	-0.3	0.5	-0.6	0.6	NA
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Ellin506		1.3	-0.2	0.5	-0.4	0.7	NA

WPS-2						1.3	0.6	0.5	1.1	0.3	NA
WS6	SC72	WCHB1-15				1.3	-0.2	0.5	-0.4	0.7	NA
WWE1	[Cloacamonae]	[Cloacamonales]	[Cloacamonaceae]	W22		1.3	-0.1	0.5	-0.2	0.8	NA
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	aquatilis	1.2	0.0	0.5	0.0	1.0	NA

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APPENDIX 4

Table S5. Table reporting the differential abundances of taxa, found in TTA samples from calves with (n = 10) and without (n = 7) lung consolidation obtained by the DESeq2 analysis. As reported in the DESeq2 support information, the "base mean" column reports the mean of normalized counts for all samples, while the "log2FoldChange" column reports the log fold change calculated for the calves without lung consolidation as compared with calves with lung consolidation, with the relative standard error in the adjacent column "lfcSE" (that is log2 fold change Standard Error). For statistical significance, *P* values are reported without (*P*value) and with adjustment (padj) for multiple testing with the false discovery rate (FDR).

Phylum	Class	Order	Family	Genus	Species	Base mean	Log2Fold Change	lfcSE	Stat	*P* value	p adj
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella	multocida	4358,5	-9,4	1,1	-8,2	2,1e-16	3,6e-14
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Mannheimia		80,8	-5,5	1,2	-4,6	4,0e-06	3,4e-04
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae			73,1	-4,4	1,0	-4,3	1,8e-05	1,0e-03
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium		9,9	-4,1	1,0	-4,1	4,1e-05	1,8e-03
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae			7,3	-3,4	1,0	-3,3	1,1e-03	3,7e-02
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus		7,6	-2,9	0,9	-3,2	1,3e-03	3,7e-02
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus		4,4	-2,6	0,8	-3,1	2,2e-03	4,7e-02
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Ureaplasma		762,4	-4,1	1,3	-3,1	2,1e-03	4,7e-02
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia		3,6	-2,4	0,8	-2,9	0,0	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides		6,1	-2,2	0,8	-2,8	0,0	0,1
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Other	Other	1428,6	3,1	1,1	2,7	0,0	0,1
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella		17,0	-2,7	1,0	-2,8	0,0	0,1
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae			3,5	-2,3	0,8	-2,7	0,0	0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]			4,2	2,1	0,8	2,7	0,0	0,1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter		2,7	2,0	0,8	2,7	0,0	0,1
Bacteroidetes	Bacteroidia	Bacteroidales				6,1	-2,3	0,9	-2,6	0,0	0,1
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium		2,5	1,7	0,7	2,6	0,0	0,1
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae			13,6	-2,7	1,0	-2,6	0,0	0,1
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	succinogenes	2,6	-2,0	0,8	-2,5	0,0	0,1
Firmicutes	Bacilli	Bacillales	Planococcaceae	Planomicrobium		2,3	-1,8	0,8	-2,3	0,0	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas		4,8	-2,1	0,9	-2,3	0,0	0,2

Actinobacteria	Actinobacteria	Actinomycetales	Yaniellaceae	Yaniella		2,2	-1,7	0,8	-2,2	0,0	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	eggerthii	2,1	-1,6	0,8	-2,1	0,0	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides		2,1	-1,6	0,7	-2,2	0,0	0,2
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]		2,2	-1,7	0,8	-2,1	0,0	0,2
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus		2,2	-1,7	0,8	-2,1	0,0	0,2
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium	prausnitzii	2,2	-1,5	0,7	-2,1	0,0	0,2
Proteobacteria	Alphaproteobacteria	RF32				2,2	-1,7	0,8	-2,1	0,0	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Aurantimonadaceae			2,2	1,5	0,7	2,1	0,0	0,2
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Other	Other	2,6	-1,6	0,8	-2,1	0,0	0,2
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae			2,1	-1,6	0,8	-2,1	0,0	0,2
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Mycetocola		1,8	1,2	0,6	2,0	0,1	0,2
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	stercorea	2,6	-1,5	0,8	-1,9	0,1	0,2
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella		2,2	-1,5	0,8	-1,9	0,1	0,2
Firmicutes	Clostridia	Clostridiales	Other	Other	Other	4,1	-1,5	0,8	-1,9	0,1	0,2
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	marcusii	2,0	-1,5	0,8	-2,0	0,0	0,2
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	Other	2,0	-1,5	0,8	-2,0	0,0	0,2
Proteobacteria	Deltaproteobacteria	GMD14H09				2,4	-1,5	0,8	-1,9	0,1	0,2
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Other	Other	632,4	-2,6	1,3	-1,9	0,1	0,2
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae			4,3	-1,6	0,8	-1,9	0,1	0,2
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter		2,7	-1,5	0,8	-2,0	0,0	0,2
TM7	TM7-3	CW040	F16			2,1	-1,4	0,7	-1,9	0,1	0,2
[Thermi]	Deinococci	Deinococcales	Deinococcaceae	Deinococcus		1,6	1,2	0,7	1,9	0,1	0,2
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Clavibacter	Other	2,1	-1,4	0,8	-1,8	0,1	0,2
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	neonatale	2,0	-1,5	0,8	-1,8	0,1	0,2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia		2,1	1,4	0,7	1,9	0,1	0,2
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Other	Other	1,8	1,2	0,7	1,8	0,1	0,2
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leucobacter		1,8	1,2	0,6	1,8	0,1	0,2
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Peptoniphilus		1,9	-1,4	0,8	-1,8	0,1	0,2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	echinoides	2,1	-1,4	0,8	-1,8	0,1	0,2
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	viridiflava	2,3	-1,4	0,8	-1,8	0,1	0,2

Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae			1,9	-1,4	0,8	-1,8	0,1	0,2
Firmicutes	Bacilli	Bacillales	Bacillaceae			8,8	-1,2	0,7	-1,7	0,1	0,3
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Helcococcus		2,0	-1,3	0,8	-1,8	0,1	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae			2,4	-1,3	0,8	-1,7	0,1	0,3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter		3,1	-1,2	0,7	-1,7	0,1	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri	3,6	-1,3	0,8	-1,7	0,1	0,3
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Wautersiella		1,5	1,1	0,6	1,7	0,1	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae			1,8	-1,3	0,8	-1,7	0,1	0,3
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes		1,9	1,2	0,7	1,7	0,1	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Beutenbergiaceae	Other	Other	1,8	1,0	0,6	1,6	0,1	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	Other	Other	1,8	-1,3	0,8	-1,6	0,1	0,3
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	Other	1,6	-1,1	0,6	-1,6	0,1	0,3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	sanguinis	4,0	-1,3	0,8	-1,6	0,1	0,3
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	Other	1,6	-1,1	0,7	-1,6	0,1	0,3
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	[Eubacterium]	biforme	1,6	-1,1	0,7	-1,6	0,1	0,3
Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Cardiobacterium		1,7	-1,2	0,8	-1,6	0,1	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	RF16			1,6	0,9	0,6	1,5	0,1	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	CF231		2,9	-1,1	0,7	-1,5	0,1	0,3
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium		1,8	1,0	0,6	1,5	0,1	0,3
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae			10,9	-1,3	0,8	-1,5	0,1	0,3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	Other	1,6	-1,1	0,7	-1,5	0,1	0,3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	stutzeri	1,6	-1,1	0,7	-1,5	0,1	0,3
TM7	TM7-1					1,5	1,1	0,7	1,6	0,1	0,3
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter		1,9	-1,0	0,7	-1,5	0,1	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium		2,0	-1,1	0,7	-1,5	0,1	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Micrococcus		1,6	-0,9	0,6	-1,5	0,1	0,3
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae			1,6	-1,1	0,7	-1,5	0,1	0,3
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter		2,6	1,1	0,7	1,5	0,1	0,3
Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	Turicibacter		2,2	1,0	0,7	1,5	0,1	0,3
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia		1,6	-1,1	0,7	-1,5	0,1	0,3

[Thermi]	Deinococci	Thermales	Thermaceae	Meiothermus		1,6	-1,1	0,7	-1,5	0,1	0,3
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae			2,1	-1,0	0,7	-1,4	0,2	0,3
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae			1,5	-1,0	0,7	-1,4	0,2	0,3
Bacteroidetes	Cytophagia	Cytophagales	Cyclobacteriaceae			1,5	-1,0	0,7	-1,4	0,2	0,3
Cyanobacteria	Oscillatoriophycideae	Chroococcales	Xenococcaceae	Chroococcidiopsis		1,5	-1,0	0,7	-1,4	0,2	0,3
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Other	Other	5,4	-1,1	0,8	-1,4	0,2	0,3
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Succiniclasticum		1,9	-1,0	0,7	-1,4	0,2	0,3
Proteobacteria	Gammaproteobacteria	Alteromonadales	211ds20			1,5	-1,0	0,7	-1,4	0,2	0,3
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	yabuuchiae	1,6	0,8	0,6	1,3	0,2	0,4
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae			2,9	0,9	0,7	1,3	0,2	0,4
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella		2,2	-0,9	0,7	-1,3	0,2	0,4
Firmicutes	Clostridia	Clostridiales				18,2	-1,1	0,9	-1,3	0,2	0,4
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Myroides		1,8	-0,8	0,7	-1,2	0,2	0,4
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Other	Other	1,9	-0,8	0,6	-1,2	0,2	0,4
Other	Other	Other	Other	Other	Other	22,8	-1,1	0,9	-1,2	0,2	0,4
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Mycoplasma		45445,7	-1,0	0,8	-1,2	0,2	0,4
Firmicutes	Bacilli	Bacillales	Planococcaceae	Solibacillus		1,7	-0,8	0,7	-1,2	0,2	0,4
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium		2,8	-0,8	0,7	-1,1	0,3	0,4
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum		1,7	-0,8	0,7	-1,1	0,3	0,5
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae			1,5	0,7	0,6	1,1	0,3	0,5
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	adhaesivum	2,4	-0,7	0,7	-1,0	0,3	0,5
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus	psychrophilus	1,6	0,6	0,6	1,0	0,3	0,5
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae			2,3	0,7	0,7	1,0	0,3	0,5
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Other	Other	1,6	0,6	0,6	1,0	0,3	0,5
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Dyadobacter		2,2	-0,7	0,7	-1,0	0,3	0,5
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium		4,8	-0,8	0,8	-1,0	0,3	0,5
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]		1,8	0,6	0,6	1,0	0,3	0,5
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Cloacibacterium		2,4	-0,7	0,7	-1,0	0,3	0,5
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas		1,9	-0,6	0,7	-0,9	0,4	0,6
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus		1,7	-0,6	0,6	-0,9	0,4	0,6

Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium		1,5	-0,6	0,6	-0,9	0,4	0,6
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus		1,6	0,5	0,6	0,9	0,4	0,6
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae			1,9	-0,6	0,7	-0,9	0,4	0,6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae			5,0	-0,7	0,8	-0,9	0,4	0,6
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella		19,4	-0,8	1,0	-0,9	0,4	0,6
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio		2,4	-0,6	0,7	-0,8	0,4	0,6
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium		2,5	-0,6	0,7	-0,8	0,4	0,6
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus		3,3	0,6	0,7	0,8	0,4	0,6
Proteobacteria	Alphaproteobacteria	Rhizobiales				2,0	0,5	0,7	0,8	0,4	0,6
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Other	Other	1,6	-0,5	0,6	-0,8	0,4	0,6
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Parvimonas		1,6	-0,5	0,6	-0,8	0,4	0,6
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae			1,5	0,5	0,6	0,8	0,5	0,6
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	equorum	2,2	0,5	0,7	0,7	0,5	0,6
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	lwoffii	5,0	-0,6	0,8	-0,7	0,5	0,6
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae			7,8	-0,7	1,0	-0,7	0,5	0,6
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae			2,5	-0,5	0,8	-0,7	0,5	0,7
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus		2,0	-0,5	0,7	-0,7	0,5	0,7
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae			2,6	-0,5	0,8	-0,7	0,5	0,7
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio		3,0	0,5	0,7	0,7	0,5	0,7
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus		1,7	-0,4	0,6	-0,6	0,5	0,7
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter		4,0	-0,5	0,8	-0,6	0,5	0,7
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae			2,3	-0,4	0,7	-0,6	0,5	0,7
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	producta	1,6	0,4	0,6	0,6	0,6	0,7
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae			1,5	-0,3	0,6	-0,6	0,6	0,7
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter		2,1	0,4	0,7	0,6	0,6	0,7
Cyanobacteria	Chloroplast	Streptophyta				1,8	0,3	0,6	0,5	0,6	0,7
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae			1,6	-0,3	0,6	-0,5	0,6	0,7
Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	fascians	2,2	0,4	0,7	0,5	0,6	0,7
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium		23,9	0,4	0,8	0,5	0,6	0,7
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	diminuta	1,9	0,3	0,7	0,5	0,6	0,7

Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium		9,7	-0,4	0,8	-0,5	0,6	0,8
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus	capsulatus	3,5	0,4	0,8	0,5	0,6	0,8
Firmicutes	Clostridia	Clostridiales	Clostridiaceae			6,6	0,3	0,8	0,4	0,7	0,8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Other	Other	1,5	0,3	0,6	0,4	0,7	0,8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Other	Other	1,5	0,3	0,6	0,4	0,7	0,8
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae			2,1	-0,3	0,7	-0,4	0,7	0,8
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus		1,7	0,3	0,6	0,4	0,7	0,8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	leguminosarum	1,6	-0,2	0,6	-0,4	0,7	0,8
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas		2,2	0,2	0,7	0,3	0,7	0,8
Bacteroidetes	Bacteroidia	Bacteroidales	S24-7			1,7	-0,2	0,6	-0,3	0,7	0,8
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Phascolarctobacterium		2,5	0,2	0,7	0,3	0,8	0,8
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea		3,8	0,2	0,8	0,3	0,8	0,8
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae			1,7	0,2	0,6	0,3	0,8	0,9
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Delftia		34,4	-0,2	0,8	-0,2	0,8	0,9
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	5-7N15		2,3	0,2	0,7	0,2	0,8	0,9
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia		2,5	-0,1	0,7	-0,2	0,8	0,9
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae			3,0	0,2	0,8	0,2	0,8	0,9
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae			2,1	-0,1	0,7	-0,2	0,8	0,9
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus		5,2	-0,1	0,7	-0,1	0,9	0,9
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	Dietzia		2,2	0,1	0,7	0,1	0,9	0,9
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae			766,2	0,2	1,3	0,1	0,9	0,9
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae			1,8	0,1	0,6	0,1	0,9	0,9
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae			17,8	-0,1	0,9	-0,1	0,9	1,0
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas		13,9	0,0	0,6	-0,1	1,0	1,0
Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae			1,6	0,0	0,6	-0,1	1,0	1,0
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium	acnes	2,6	0,0	0,7	0,0	1,0	1,0
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia		5,0	0,0	0,9	0,0	1,0	1,0
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter		3,5	0,0	0,8	0,0	1,0	1,0
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae			3,0	0,0	0,8	0,0	1,0	1,0

Euryarchaeota	Thermoplasmata	E2	[Methanomassiliicoccaceae]	vadinCA11		1,3	-0,7	0,6	-1,1	0,3	NA
Actinobacteria	Acidimicrobiia	Acidimicrobiales				1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111			1,1	0,3	0,6	0,5	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae			1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces		1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Trueperella		1,2	-0,5	0,6	-0,8	0,5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Varibaculum		1,1	0,3	0,6	0,5	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinopolysporaceae			1,1	-0,3	0,6	-0,4	0,7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium		1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Other	Other	1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae			1,4	0,2	0,6	0,3	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Demequina		1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	variabile	1,2	0,4	0,6	0,7	0,5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium	conglomeratum	1,4	0,3	0,6	0,4	0,7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Other	Other	1,2	-0,5	0,6	-0,8	0,5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Kineococcus	Other	1,4	-0,8	0,7	-1,1	0,3	NA
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	Kineococcus		1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae			1,3	0,4	0,6	0,6	0,5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agrococcus		1,1	-0,3	0,6	-0,4	0,7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Cryocola		1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Curtobacterium		1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Pseudoclavibacter	bifida	1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Rathayibacter	caricis	1,3	-0,1	0,6	-0,1	0,9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Other	Other	1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Kocuria	rhizophila	1,2	-0,5	0,6	-0,8	0,5	NA
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	dentocariosa	1,0	0,0	0,7	0,0	1,0	NA
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium		1,2	-0,1	0,6	-0,2	0,9	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae			1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Aeromicrobium		1,2	-0,4	0,6	-0,6	0,6	NA
Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae			1,2	0,4	0,6	0,7	0,5	NA

Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	Luteococcus		1,1	-0,3	0,6	-0,4	0,7	NA
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia		1,0	0,0	0,7	0,0	1,0	NA
Actinobacteria	Actinobacteria	Actinomycetales	Sanguibacteraceae	Sanguibacter		1,1	0,2	0,7	0,2	0,8	NA
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces		1,2	-0,5	0,6	-0,8	0,5	NA
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Other	1,1	-0,1	0,7	-0,2	0,8	NA
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium		1,4	-0,8	0,6	-1,3	0,2	NA
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	pseudolongum	1,1	0,2	0,7	0,2	0,8	NA
Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	Patulibacter		1,1	-0,1	0,7	-0,2	0,8	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Other	1,1	-0,3	0,6	-0,4	0,7	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	barnesiae	1,1	0,3	0,6	0,5	0,6	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	coprophilus	1,3	-0,3	0,6	-0,5	0,6	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae			1,0	0,0	0,7	0,0	1,0	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	endodontalis	1,3	-0,7	0,6	-1,1	0,3	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	intermedia	1,2	-0,4	0,6	-0,6	0,6	NA
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae			1,3	-0,6	0,6	-0,9	0,4	NA
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	YRC22		1,1	0,0	0,6	0,0	1,0	NA
Bacteroidetes	Bacteroidia	Bacteroidales	p-2534-18B5			1,1	-0,3	0,6	-0,4	0,7	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Rudanella		1,1	-0,1	0,7	-0,2	0,8	NA
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Spirosoma		1,0	0,0	0,7	0,0	1,0	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae			1,1	-0,3	0,6	-0,4	0,7	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga		1,1	0,3	0,6	0,5	0,6	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Myroides	odoratimimus	1,3	0,7	0,6	1,2	0,2	NA
Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Ornithobacterium		1,5	-0,9	0,7	-1,3	0,2	NA
Bacteroidetes	Sphingobacteriia	Sphingobacteriales				1,2	-0,4	0,6	-0,6	0,6	NA
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	cryoconitis	1,3	0,6	0,6	1,0	0,3	NA
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium		1,2	-0,4	0,6	-0,6	0,6	NA
Bacteroidetes	[Saprospirae]	[Saprospirales]				1,1	-0,1	0,7	-0,2	0,8	NA
Chlamydiae	Chlamydiia	Chlamydiales	Chlamydiaceae	Chlamydia	Other	1,0	0,0	0,7	0,0	1,0	NA
Chloroflexi	Thermomicrobia	JG30-KF-CM45				1,2	-0,1	0,6	-0,2	0,9	NA
Cyanobacteria	4C0d-2	YS2				1,3	-0,6	0,6	-0,9	0,4	NA

Cyanobacteria	Chloroplast	Stramenopiles				1,3	-0,2	0,6	-0,3	0,8	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Other	Other	1,2	-0,1	0,6	-0,2	0,9	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Other	1,3	-0,3	0,6	-0,5	0,6	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	cereus	1,5	-0,7	0,6	-1,1	0,3	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	flexus	1,2	-0,4	0,6	-0,6	0,6	NA
Firmicutes	Bacilli	Bacillales	Bacillaceae	Natronobacillus		1,1	-0,3	0,6	-0,4	0,7	NA
Firmicutes	Bacilli	Bacillales	Planococcaceae	Rummeliibacillus		1,5	1,0	0,6	1,6	0,1	NA
Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina		1,1	0,2	0,7	0,2	0,8	NA
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus		1,2	-0,4	0,6	-0,6	0,6	NA
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Salinicoccus		1,3	-0,7	0,6	-1,1	0,3	NA
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Other	1,0	0,0	0,7	0,0	1,0	NA
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	sciuri	1,4	0,7	0,6	1,1	0,3	NA
Firmicutes	Bacilli	Lactobacillales				1,1	-0,1	0,7	-0,2	0,8	NA
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Trichococcus		1,4	-0,3	0,6	-0,5	0,6	NA
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus		1,1	-0,1	0,7	-0,2	0,8	NA
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus		1,3	0,7	0,6	1,2	0,2	NA
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	brevis	1,1	0,3	0,6	0,5	0,6	NA
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc		1,1	0,2	0,7	0,2	0,8	NA
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus		1,1	0,2	0,7	0,2	0,8	NA
Firmicutes	Clostridia	Clostridiales	Christensenellaceae			1,0	0,0	0,7	0,0	1,0	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Other	Other	1,1	-0,1	0,7	-0,2	0,8	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Proteiniclasticum		1,3	0,7	0,6	1,2	0,2	NA
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Tindallia_Anoxynatronum		1,1	-0,3	0,6	-0,4	0,7	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	Other	1,3	0,2	0,6	0,3	0,8	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia		1,2	0,2	0,6	0,3	0,8	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	faecis	1,1	-0,1	0,7	-0,2	0,8	NA
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia		1,3	-0,6	0,6	-0,9	0,4	NA
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Peptococcus		1,1	0,2	0,7	0,2	0,8	NA
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Filifactor		1,3	-0,7	0,6	-1,1	0,3	NA
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus	Other	1,1	-0,3	0,6	-0,4	0,7	NA

Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Other	Other	1,2	-0,4	0,6	-0,6	0,6	NA
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira		1,3	0,7	0,6	1,2	0,2	NA
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	bromii	1,4	0,8	0,6	1,3	0,2	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae			1,2	-0,4	0,6	-0,6	0,6	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Anaerovibrio		1,1	-0,3	0,6	-0,4	0,7	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas		1,0	0,0	0,7	0,0	1,0	NA
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar	1,4	-0,8	0,6	-1,2	0,2	NA
Firmicutes	Clostridia	Clostridiales	[Acidaminobacteraceae]	Guggenheimella		1,3	0,2	0,6	0,3	0,8	NA
Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]			1,0	0,0	0,7	0,0	1,0	NA
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]			1,4	0,9	0,7	1,4	0,2	NA
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Anaerococcus		1,1	0,3	0,6	0,5	0,6	NA
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Finegoldia		1,0	0,0	0,7	0,0	1,0	NA
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	GW-34		1,1	0,2	0,7	0,2	0,8	NA
Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Tissierella_Soehngenia		1,2	-0,5	0,6	-0,8	0,5	NA
Firmicutes	Clostridia	Natranaerobiales				1,1	0,3	0,6	0,5	0,6	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	p-1630-c5	1,1	0,2	0,7	0,2	0,8	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus		1,4	-0,5	0,6	-0,8	0,4	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Erysipelothrix		1,3	-0,7	0,6	-1,1	0,3	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	RFN20		1,1	-0,3	0,6	-0,4	0,7	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Sharpea	p-3329-23G2	1,1	-0,1	0,7	-0,2	0,8	NA
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	p-75-a5		1,2	-0,4	0,6	-0,6	0,6	NA
OD1						1,1	0,3	0,6	0,5	0,6	NA
Planctomycetes	Phycisphaerae	Phycisphaerales				1,1	-0,1	0,7	-0,2	0,8	NA
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae			1,1	0,3	0,6	0,5	0,6	NA
Proteobacteria	Alphaproteobacteria					1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			1,3	0,6	0,6	1,0	0,3	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae			1,3	0,3	0,6	0,5	0,7	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes		1,1	0,3	0,6	0,5	0,6	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae			1,4	-0,8	0,6	-1,3	0,2	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1,1	0,2	0,7	0,2	0,8	NA

Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium		1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Other	Other	1,1	0,2	0,7	0,2	0,8	NA
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus		1,3	0,5	0,6	0,8	0,4	NA
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter		1,2	0,5	0,6	0,9	0,4	NA
Proteobacteria	Alphaproteobacteria	Rickettsiales	mitochondria	Other	Other	1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Alphaproteobacteria	Sphingomonadales				1,4	-0,8	0,6	-1,3	0,2	NA
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium		1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	capsulatum	1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	wittichii	1,1	0,2	0,7	0,2	0,8	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter		1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Lautropia		1,1	-0,1	0,7	-0,2	0,8	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae			1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas		1,3	-0,7	0,6	-1,1	0,3	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Tepidimonas		1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Cupriavidus	Other	1,1	-0,1	0,7	-0,2	0,8	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium		1,1	0,3	0,6	0,5	0,6	NA
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Ralstonia		1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae			1,4	-0,8	0,6	-1,3	0,2	NA
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Propionivibrio		1,1	-0,1	0,7	-0,2	0,8	NA
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Thauera		1,1	-0,1	0,7	-0,2	0,8	NA
Proteobacteria	Deltaproteobacteria	Spirobacillales				1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	cryaerophilus	1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter		1,2	-0,4	0,6	-0,6	0,6	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales				1,1	0,3	0,6	0,5	0,6	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Other	Other	1,2	0,5	0,6	0,9	0,4	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae			1,1	0,2	0,7	0,2	0,8	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	BD2-13		1,3	-0,6	0,6	-0,9	0,4	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Candidatus Endobugula		1,2	-0,1	0,6	-0,2	0,9	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Cellvibrio		1,3	-0,7	0,6	-1,1	0,3	NA

Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Marinobacter		1,1	0,2	0,7	0,2	0,8	NA
Proteobacteria	Gammaproteobacteria	Alteromonadales	[Chromatiaceae]	Rheinheimera		1,1	-0,1	0,7	-0,2	0,8	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Other	Other	1,1	0,2	0,7	0,2	0,8	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia		1,4	-0,6	0,6	-1,0	0,3	NA
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Morganella	morganii	1,2	-0,4	0,6	-0,6	0,6	NA
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas		1,0	0,0	0,7	0,0	1,0	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Bibersteinia	Other	1,1	-0,3	0,6	-0,4	0,7	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae	1,0	0,0	0,7	0,0	1,0	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella	Other	1,2	-0,5	0,6	-0,8	0,5	NA
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella		1,2	-0,4	0,6	-0,6	0,6	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Other	Other	Other	1,1	-0,1	0,7	-0,2	0,8	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	johnsonii	1,3	0,0	0,6	0,1	0,9	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	schindleri	1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter		1,2	0,4	0,6	0,7	0,5	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Other	1,2	-0,4	0,6	-0,6	0,6	NA
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	1,3	0,6	0,6	1,0	0,3	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Nevskia		1,1	0,0	0,6	0,0	1,0	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae			1,0	0,0	0,7	0,0	1,0	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas		1,3	-0,6	0,6	-0,9	0,4	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	Other	1,5	-0,9	0,7	-1,3	0,2	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Rhodanobacter		1,1	0,3	0,6	0,5	0,6	NA
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Other	1,0	0,0	0,7	0,0	1,0	NA
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema		1,2	0,2	0,6	0,3	0,8	NA
TM6	SJA-4					1,1	-0,1	0,7	-0,2	0,8	NA
TM7	TM7-3					1,2	0,4	0,6	0,7	0,5	NA
TM7	TM7-3	EW055				1,1	0,2	0,7	0,2	0,8	NA
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma		1,1	0,2	0,7	0,2	0,8	NA
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	laidlawii	1,2	0,3	0,6	0,5	0,6	NA
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma		1,2	0,0	0,6	0,1	1,0	NA
Tenericutes	Mollicutes	RF39				1,1	-0,3	0,6	-0,4	0,7	NA

4074 **APPENDIX 5**

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4076 **Table S6:** Total, individual and group treatment nADD are reported as mean ± standard deviation (sd) (median, max) for each parameter of the

4077 categorical variables analyzed.

Parameter	Category	n° farms	Percent	Mean of nADD ± sd (median, max)		
				Total treatment	Single treatment	Group treatment
Purchasing at least the 10% of females	Yes	10	38.5%	3.2 ± 2.2 (3.1, 8.3)	1.2 ± 0.8 (1.2, 2.6)	2 ± 2 (1.6, 6.8)
	No	16	61.5%	2.9 ± 2 (2.5, 8.2)	1.4 ± 0.7 (1.5, 3)	1.5 ± 1.8 (0.8, 6)
Maximal weight difference between average animals' weight at arrival (kg)	<50	7	26.9%	3.1 ± 2.6 (2.6, 8.3)	1.3 ± 0.9 (1.6, 3)	1.8 ± 2.4 (1, 6.8)
	50-100	13	50%	3.2 ± 2.3 (2.6, 8.2)	1.3 ± 0.7 (1.4, 2.6)	1.9 ± 2 (1.2, 6)
	>100	6	23.1%	2.4 ± 1.2 (2.6, 3.8)	1.2 ± 0.5 (1.2, 1.7)	1.2 ± 1 (1, 2.3)
Pre-arrival health information	Yes	1	3.8%	6.8	1.6	5.4
	No	25	96.2%	2.8 ± 2 (2.6, 8.3)	1.2 ± 0.7 (1.4, 3)	1.6 ± 1.8 (1, 6.8)
Thorough physical examination at arrival	Yes	8	30.8%	2.5 ± 1.1 (2.5, 4.4)	0.9 ± 0.4 (0.8, 1.6)	1.6 ± 1.2 (1.5, 3.9)
	No	18	69.2%	3.2 ± 2.4 (2.8, 8.3)	1.4 ± 0.7 (1.5, 3)	1.8 ± 2.1 (0.9. 6.8)
vaccination against bacteria	Yes	23	88.5%	3 ± 2.2 (2.6, 8.3)	1.3 ± 0.7 (1.4, 3)	1.7 ± 2 (1, 6.8)
	No	3	11.5%	2.7 ± 1.2 (2.3, 4.1)	1.2 ± 0.7 (1.6, 1.6)	1.5 ± 0.9 (1.2, 2.4)
Interval longer than 1 days between arrival and vaccination	Yes	10	38.5%	2.8 ± 2.3 (2.1, 8.3)	1.1 ± 0.8 (0.9, 3)	1.7 ± 2.2 (1.1, 6.8)
	No	16	61.5%	3.1 ± 2.1 (2.9, 8.2)	1.4 ± 0.6 (1.5, 2.6)	1.7 ± 1.7 (1.1, 6)
Parasiticides at arrival	Yes	23	88.5%	2.7 ± 1.9 (2.3, 8.3)	1.2 ± 0.7 (1.1, 3)	1.5 ± 1.7 (0.8, 6.8)
	No	3	11.5%	5.3 ± 2.7 (4.1, 8.2)	1.9 ± 0.3 (1.7, 2.2)	3.3 ± 2.4 (2.4, 6)
Regularly prophylactic treatment at arrival	Yes	15	57.7%	3.8 ± 2.3 (3.3, 8.3)	1.2 ± 0.6 (1.5, 2.2)	2.6 ± 2 (2, 6.8)
	No	11	42.3%	1.8 ± 1.1 (1.5, 4.4)	1.4 ± 0.9 (1.1, 3)	0.5 ± 0.6 (0.1, 1.8)
Specific diet provided for animals in the quarantine period	Yes	7	26.9%	2.1 ± 1 (2.1, 3.5)	1.1 ± 0.5 (1, 1.7)	1 ± 0.7 (0.8, 2)
	No	19	73.1%	3.3 ± 2.4 (3, 8.3)	1.3 ± 0.8 (1.5, 3)	1.9 ± 2.1 (1.2, 6.8)
Presence of a restraint cage	Yes	9	34.6%	3.5 ± 2.6 (3.1, 8.2)	1.3 ± 0.5 (1.5, 2.2)	2.2 ± 2.2 (2, 6)
	No	17	65.4%	2.7 ± 1.9 (2.6, 8.3)	1.3 ± 0.8 (1.4, 3)	1.5 ± 1.7 (0.8, 6.8)
Animals handling corridor	Yes	14	53.8%	3.1 ± 2.1 (3, 8.33)	1.3 ± 0.7 (1.5, 3)	1.9 ± 2 (1.4, 6.7)
	No	12	46.2%	2.8 ± 2.2 (2.3, 8.2)	1.3 ± 0.8 (1.4, 2.6)	1.5 ± 1.7 (0.8, 6)
Fattening group divided on the base of origins or arrival	Yes	5	19.2%	2.3 ± 1.6 (1.2, 4.4)	0.8 ± 0.6 (1, 1.5)	1.5 ± 1.6 (1, 3.9)
	No	21	80.8%	3.1 ± 2.2 (2.6, 8.3)	1.4 ± 0.7 (1.5, 3)	1.8 ± 1.9 (1.2, 6.8)
Fattening group divided on the base of weight at arrival	Yes	24	92.3%	2.9 ± 2.3 (2.3, 8.3)	1.3 ± 0.6 (1.5, 3)	1.6 ± 2 (0.8, 6.8)
	No	2	7.7%	2.4 ± 1.9 (2.4, 3.8)	0.8 ± 1 (0.8, 1.5)	1.7 ± 0.9 (1.7, 2.3)
	Yes	5	19.2%	3.6 ± 2.2 (2.6, 7)	1.2 ± 0.5 (1.1, 1.8)	2.4 ± 2.2 (1.85, 5.4)

Daily animals' checks entering the pen	No	21	80.8%	2.8 ± 2.1 (2.3, 8.3)	1.3 ± 0.7 (1.5, 3)	1.6 ± 1.8 (1, 6.8)
All BRD cases are examined by the veterinary practitioner before treatment	Yes	9	34.6%	2.1 ± 1.3 (1.5, 4.4)	1.2 ± 0.8 (1.5, 2.6)	0.9 ± 0.8 (0.8, 2.3)
	No	17	65.4%	3.4 ± 2.4 (3, 8.3)	1.3 ± 0.7 (1.4, 3)	2.1 ± 2.2 (1.9, 6.8)
The animals are moved in the infirmary locals at the first onset clinical signs	Yes	5	19.2%	2.2 ± 1.1 (1.7, 3.5)	0.8 ± 0.6 (0.8, 1.5)	1.4 ± 0.6 (1.2, 2)
	No	21	80.8%	3.6 ± 2.3 (2.6, 83)	1.4 ± 0.7 (1.5, 3)	1.8 ± 2.1 (0.8, 6.8)
Mechanical ventilation in closed locals	Yes	23	88.5%	3.2 ± 2.2 (3, 8.3)	1.3 ± 0.7 (1.5, 3)	1.8 ± 2 (1.4, 6.8)
	No	3	11.5%	1.7 ± 0.8 (1.3, 2.6)	0.8 ± 0.9 (0.5, 1.8)	0.9 ± 0.1 (0.8, 1)
Open quarantine locals	Yes	17	65.4%	2.9 ± 2.2 (2.6, 8.2)	1.2 ± 0.7 (1.1, 3)	1.7 ± 1.9 (1.2, 6)
	No	9	34.6%	3.1 ± 2.2 (2.6, 8.3)	1.3 ± 0.8 (1.6, 2.6)	1.8 ± 2 (1, 6.8)
Isolated quarantine locals	Yes	23	88.5%	3 ± 2.2 (2.6, 8.3)	1.3 ± 0.7 (1.4, 3)	1.8 ± 2 (1.2, 6.8)
	No	3	11.5%	2.5 ± 1.4 (2.6, 3.8)	1.1 ± 0.9 (1.5, 1.8)	1.4 ± 0.8 (1, 2.3)
Paddock in quarantine locals	Yes	6	23.1%	2.8 ± 2.4 (1.6, 7)	0.9 ± 0.4 (0.9, 1.6)	1.9 ± 2.2 (1, 5.4)
	No	20	76.9%	3 ± 2.1 (2.8, 8.3)	1.4 ± 0.7 (1.5, 3)	1.7 ± 1.8 (1.2, 6.8)
Open fattening locals	Yes	3	11.5%	1.5 ± 0.5 (1.3, 2.1)	1 ± 0.6 (1, 1.6)	0.5 ± 0.4 (0.5, 0.8)
	No	23	88.5%	3.2 ± 2.2 (3, 8.3)	1.3 ± 0.7 (1.5, 3)	1.9 ± 1.9 (1.4, 6.8)
Paddock in fattening locals	Yes	6	23.1%	3 ± 2.2 (2.8, 7)	1.4 ± 0.9 (1.2, 3)	1.6 ± 2.1 (0.9, 5.4)
	No	20	76.9%	3 ± 2.2 (2.5, 8.3)	1.2 ± 0.7 (1.5, 2.6)	1.8 ± 1.8 (1.1, 6.8)
Open infirmary locals	Yes	11	42.3%	2.5 ± 1.3 (2.6, 4.4)	1.2 ± 0.5 (1.5, 1.8)	1.4 ± 1.2 (0.8, 3.9)
	No	15	57.7%	3.3 ± 2.6 (2.6, 8.3)	1.3 ± 0.8 (1.4, 3)	2 ± 2.2 (1.2, 6.8)
Infirmary locals isolated from the rest of the structure	Yes	8	30.8%	2.4 ± 1.6 (2.2, 4.4)	1.2 ± 0.7 (1.1, 2.6)	1.2 ± 1.3 (1, 3.9)
	No	18	69.2%	3.2 ± 2.3 (2.6, 8.3)	1.3 ± 0.7 (1.5, 3)	1.9 ± 2.1 (1.1, 6.8)
Paddock in infirmary locals	Yes	1	3.8%	1.2	1.1	0.09
	No	25	96.2%	3.1 ± 2.1 (2.6, 8.3)	1.3 ± 0.7 (1.5 3)	1.8 ± 1.9 (1.2, 6.8)
Scraper in at least one local	Yes	13	50%	3.2 ± 2.3 (3, 8.2)	1.3 ± 0.8 (1, 3)	1.9 ± 2 (1.2, 6)
	No	13	50%	2.8 ± 2 (2.6, 8.3)	1.3 ± 0.7 (1.5, 2.6)	1.5 ± 1.7 (1, 6.8)
Scraper in quarantine locals	Yes	2	7.7%	2.3 ± 1.1 (2.3, 3.1)	0.8 ± 0.07 (0.8, 0.9)	1.5 ± 1.1 (1.5, 2.2)
	No	24	92.3%	3 ± 2.2 (2.6, 8.3)	1.3 ± 0.7 (1.5, 3)	1.7 ± 1.9 (1.1, 6.8)
Quarantine locals disinfection	Yes	24	92.3%	3.1 ± 2.2 (2.8, 8.3)	1.3 ± 0.7 (1.4, 3)	1.8 ± 1.9 (1.3, 6.8)
	No	2	7.7%	1.4 ± 1 (1.4, 2.1)	1.1 ± 0.7 (1.1, 1.6)	0.2 ± 0.3 (0.2, 0.5)
Scraper in fattening locals	Yes	12	46.2%	3.3 ± 2.4 (3.1, 8.2)	1.3 ± 0.8 (1.2, 3)	2.1 ± 2.1 (1.8, 6)
	No	14	53.8%	2.7 ± 2 (2.5, 8.3)	1.3 ± 0.7 (1.5, 2.6)	1.4 ± 1.7 (0.9, 6.8)
	Scraper	6	23.1%	3.9 ± 3.1 (2.7, 8.2)	1.2 ± 0.7 (1.1, 2.2)	2.7 ± 2.4 (1.8, 6)

Bedding removing in fattening locals	Scraper and manually	6	23.1%	2.8 ± 1.5 (3.1, 4.4)	1.4 ± 0.9 (1.2, 3)	1.4 ± 1.7 (1.1, 3.9)
	Manually	14	53.8%	2.7 ± 2 (2.5, 8.3)	1.3 ± 0.7 (1.5, 2.6)	1.4 ± 1.7 (0.9, 6.8)
Fattening locals disinfection	Yes	21	80.8%	3.2 ± 2.2 (2.6, 8.3)	1.4 ± 0.7 (1.5, 3)	1.8 ± 2 (1, 6.8)
	No	5	19.2%	2.3 ± 1.6 (1.7, 4.1)	0.9 ± 0.6 (0.7, 1.6)	1.3 ± 1.1 (1.2, 2.4)
Scraper in infirmary locals	Yes	6	23.1%	2.5 ± 2.3 (1.6, 7)	1.2 ± 0.9 (0.9, 3)	1.2 ± 2 (0.4, 5.4)
	No	20	76.9%	3.1 ± 2.1 (2.9, 8.3)	1.3 ± 0.6 (1.5, 2.6)	1.9 ± 1.8 (1.6, 6.8)
Bedding removing in infirmary locals	Scraper	3	11.5%	3.4 ± 3.1 (1.7, 7)	0.9 ± 2.5 (1.2, 5.4)	2.4 ± 2.5 (1.2, 5.4)
	Scraper and manually	3	11.5%	1.6 ± 1.2 (1.1, 3)	1.5 ± 1.3 (1, 3)	0.03 ± 0.04 (0, 0.08)
	Manually	20	77%	3.1 ± 2.1 (2.9, 8.3)	1.3 ± 0.6 (1.5, 2.6)	1.9 ± 1.8 (1.6, 6.8)
Infirmary locals disinfection	Yes	24	92.3%	3.1 ± 2.1 (2.8, 8.3)	1. ± 0.7 (1.5, 3)	1.8 ± 1.9 (1.2, 6.8)
	No	2	7.7%	1.2 ± 0.7 (1.2, 1.7)	0.6 ± 0.1 (0.6, 0.7)	0.6 ± 0.9 (0.6, 1.2)
Depopulation period in fattening locals	Yes	2	7.7%	5.4 ± (5.4, 8.2)	2 ± 0.3 (2, 2.2)	3.4 ± 3.7 (3.4, 6)
	No	24	92.3%	2.8 ± 1.9 (2.5, 8.3)	1.2 ± 0.7 (1.3, 3)	1.6 ± 1.7 (1.1, 6.8)
Depopulation period in infirmary locals	Yes	11	42.3%	2.3 ± 2 (3.1, 8.2)	1.4 ± 0.5 (1.5, 2.2)	1.9 ± 1.8 (1.9, 6)
	No	15	57.7%	2.8 ± 2.3 (2.1, 8.3)	1.2 ± 0.8 (1, 3)	1.5 ± 2 (0.8, 6.8)

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