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Congenital hydrocephalus in three sheep: Clinical, electroencephalographic and pathological features

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Abstract: The clinical, electroencephalographic and neuro-pathological features of three cases (cases 1, 2 and 3) of congenital hydrocephalus in sheep were described. The observed neurological signs reflected damage in the telencephalon and brain stem. The electroencephalogram performed in case 1 and case 2 showed different patterns: symmetric and synchronous high-voltage slow-activity in case 1, and low-voltage slow-activity in case 2. By the post-mortem examination, in all the animals, dilatation of the ventricular system, especially of the lateral ventricles, associated with a glial reaction surrounding the dilated ventricles was observed. Only in case 3, a monolateral meningeal thickening at the left cerebellopontine angle seemed to be responsible for the obstructive hydrocephalus. In the other two brains (case 1 and 2), no potential anatomical cause for the hydrocephalus were detected, even if, in case 2, a compensatory form was not excluded due to the moderate hypoplasia of the cerebrum and the presence of the non-suppurative inflammation. The results of this work provide a contribution to the EEG characterisation in ovine hydrocephalus cases; nevertheless further multidisciplinary studies of a larger number of sheep could permit to better characterise the EEG pattern in ovine hydrocephalus cases.

Keywords: brain; electroencephalogram; malformation; neuropathology; sheep

Hydrocephalus is the most common congenital disorder in lambs, although its prevalence in the ovine species is low (Dennis 1975). Congenital hydrocephalus can be caused by genetic factors, *in utero* exposure to infectious agents (*Toxoplasma gondii*, Akabane virus, Cache Valley virus, Blue tongue and Border disease viruses), toxic substances (i.e., *Veratrum californicum*), or nutritional deficiencies (copper) (Binns et al. 1965; Haughey et al. 1988; Edwards et al. 1989; VanderKop 1991; Woods and Anderson 1992; Adjou et al. 2007; Potter et al. 2010).

The term hydrocephalus indicates an increase in the cerebrospinal fluid (CSF) volume within the ventricular system (internal hydrocephalus) or in the subarachnoid space (external hydrocephalus), due to an imbalance between the production, flow and absorption of CSF. There are two main causes of congenital hydrocephalus: a loss of cerebral parenchyma (compensatory or *ex vacuo*

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hydrocephalus) or an intraventricular obstruction of the CSF flow (non-communicating or obstructive hydrocephalus). Compensatory hydrocephalus is generally due to a severe cerebral hypoplasia/hydranencephaly frequently caused by *in utero* viral infections. In this form of hydrocephalus, the CSF volume increases to compensate the loss of the parenchyma and the intracranial pressure is normal (normotensive hydrocephalus). Non-communicating hydrocephalus is due to an intraventricular obstruction to the CSF flow, preventing communication between the ventricular system and the subarachnoid space. In this case, the CSF pressure increases (hypertensive hydrocephalus) expanding the ventricular system. The lateral ventricles are the most susceptible to the expansion, which result in parenchymal ischemia and atrophy (de Lahunta and Glass 2009).

In congenital hydrocephalus, the increase in the intracranial volume occurs before the sutures of the calvaria have closed, allowing the enlargement of the cranial cavity. Affected animals often show neurological deficits associated with a prominent dome-shaped skull, open sutures and persistent fontanelles. They rarely survive and often are stillborn or die soon after birth. Moreover, there is a high incidence of dystocia due to the enlarged head (Miller 1993; Pritchard et al. 1994; de Lahunta and Glass 2009; Smith and George 2009).

The diagnosis of hydrocephalus is based on clinical features, imaging – ultrasonography, computed tomography (CT), magnetic resonance (MR) – and electrodiagnostic (electroencephalography) examinations of the brain. Electroencephalography is a non-invasive investigation with no adverse effects, it is not cost-prohibitive as the CT or MR and it allows one to assess the impact of the hydrocephalus on the electrical activity of the brain. Until now, electroencephalographic patterns in congenital hydrocephalus have been well described in human medicine, and only few studies have been reported in dogs (Prynn and Redding 1968; Klemm and Hall 1971; Klemm and Hall 1972; Okurowska-Zawada et al. 2007; Armasu et al. 2014). To our knowledge, the electroencephalographic patterns in sheep affected by hydrocephalus have been described only in one case (Masucci et al. 2012).

The aim of this study is to describe the clinical, electroencephalographic and neuro-pathological features of three cases of congenital hydrocephalus in ovine species.

Case description

CASE HISTORIES AND CLINICAL EXAMINATIONS

Three sheep with neurological static signs recognised shortly after birth were subject to a neurological examination, an electroencephalogram (EEG) and a neuro-pathologic examination (cases 1, 2 and 3). One clinically healthy sheep was submitted for an EEG to obtain a control record (case 4).

ELECTROENCEPHALOGRAMS

An electroencephalogram (EEG) was performed during the clinical examination in cases 1, 2 and 4.

The EEG was recorded using a digital electroencephalograph (Medelec Profile; Oxford Instruments Medical System Division, Surrey, UK; www.oxfordinstruments.com). Six subdermal needle electrodes (MedelecAccessories; Oxford Instruments Medical Systems, Surrey, UK) were inserted subcutaneously on the skull. The electrode arrangement consisted of four exploring electrodes placed on the right (F4) and left (F3) frontoparietal areas and over the right (P4) and left (P3) occipital areas; one reference electrode (Cz) was placed over the vertex and one ground electrode was located over the external occipital protuberance (Figure 1). Therefore, it was possible to obtain four referential derivations (the four areas of the cerebral hemispheres against the vertex leads: F3Cz, F4Cz, P3Cz, P4Cz) and four bipolar derivations: left and right hemispheric leads (frontal to occipital: F3P3, F4P4),

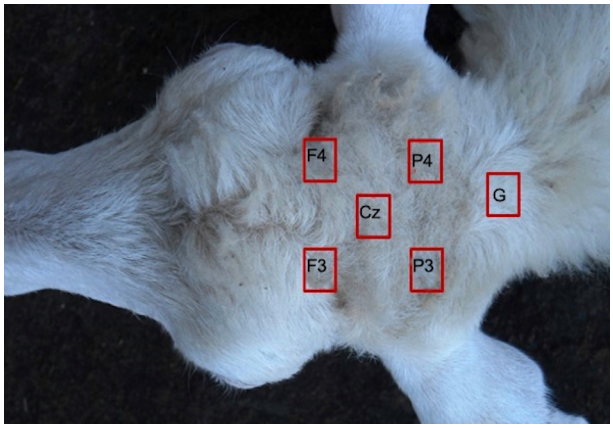

Figure 1. Electrode arrangement: Exploring electrodes (F4, F3, P4, P3), reference electrode (Cz) and ground electrode (G)

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trans-frontal leads (F3P3) and trans-occipital leads (P3P4) (Redding 1987). The EEG examination settings were as follows: chart speed 30 mm/sec, sensitivity 50 μV/cm, high pass-filter 0.5 Hz, low pass-filter 70 Hz. The electrode resistance was kept below 20 kΩ. The recordings were made in a quiet and dim lit room on un-sedated animals, manually restrained, which lasted 20 minutes.

During the EEG examination, the animals were handled according to their biological characteristics to ensure the animal's welfare. All the procedures were designed to minimise discomfort, distress and pain to the animals. The animal care and treatment were conducted in accordance with both institutional guidelines and international laws and policies. At the end of the studies, the animals returned to the herd of origin according to Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes.

All the EEGs were evaluated visually as well as quantitatively, by frequency spectral analysis. The background activity and possible superimposed transient activity were analysed by a visual evaluation, placing special emphasis on the detection of artefacts. For all the animals, six replications of 10-second artifact-free epochs were randomly selected from parts of the EEG corresponding to a relaxed waking state to analyse one minute of the recording and to perform the quantitative analysis. The spectral bands delta (0.5–3.9 Hz), theta (4–7.9 Hz), alfa (8–12.9 Hz) and beta (13–30 Hz) were calculated and expressed as the relative power (Saito et al. 2005; Cwynar et al. 2014). The median values of the relative power of each frequency band, obtained in the six epochs, was calculated for each of the eight derivations described above. The amplitude of 10 waves was measured for each derivation at the beginning of each epoch.

The statistical analyses of the EEG patterns were performed using the software GraphPad Prism v6 (GraphPad Software Inc, USA). The D'Agostino-Pearson normality test was used to test the normality of the data distribution before the statistical analyses. The Kruskal-Wallis test (post hoc test: Dunn's Multiple Comparison test) was performed to compare: 1) the delta, theta, alfa and beta spectral bands relative power between the three animals; 2) the delta, theta, alfa and beta spectral bands relative power between the eight derivations for each animal; 3) the wave voltage between the three animals. *P*-values ≤ 0.05 were considered significant.

ANATOMO-HISTOPATHOLOGICAL EVALUATION OF THE BRAINS

After death or the slaughtering of the animals, the brains were macroscopically evaluated and subsequently fixed in 10% neutral buffered formalin for a histological examination. Coronal slices of the cerebrum and cerebellum were paraffin embedded and sectioned to obtain 3 μm slide sections stained with haematoxylin and eosin (H&E).

RESULTS

Case histories and clinical examinations

Case 1. A 6-month-old female Comisana breed of sheep, the twin of another stillborn, showed neurological signs immediately after delivery. At birth, failure to bond to the dam and to the rest of the flock, a diminished learning ability, partial failure of suckling and drinking, and bruxism were detected. At six months of age, by the clinical examination, poor body development, a slight enlargement of the calvarium, fixed ventro-lateral strabismus (Figure 2A), postural reactions deficits of all the limbs and bilateral failure of the menace reaction were observed.

Considering the life conditions of the animal as being compatible with the well-being, according to the owner, the animal was not submitted to euthanasia to monitor the disease. The sheep died at one year of age do to bloat. The course of the disease was stable for all its life. Neither pathological data nor samples were recorded/collected about the stillborn twin.

Case 2. A female Comisana breed, 28 month-old sheep remained isolated from the flock, showed a compulsive gait, circling, and alternated phases of depression and excitement after a few months from birth. By the neurological examination, wide head excursions to either side, delayed postural reactions in all the limbs, bilaterally reduced menace response, bilateral mydriasis, bilateral absence of both pupillary light response and physiological nystagmus were detected. The shape and size of the skull were not well appreciable due to the presence of horns with a wide base of attachment. The course of the symptoms was stable for all its life and, according to the owner, the animal was slaughtered at 30 months of age.

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Figure 2. (A) Case 1: Fixed ventro-lateral strabismus. (B) Case 3: Absence of placing reaction in right thoracic limb

Case 3. A male, Comisana breed, 20 day-old lamb showed alterations in its gait and behavior at birth. By the neurological examination, compulsive pacing with circling to the left, non-constant mild opisthotonos, mild disorientation and absence of postural reactions in all the limbs were detected (Figure 2B). The skull was normal. Due to the life conditions being incompatible with its well-being, according to the owner, the lamb was slaughtered at 2 months of age.

Case 4. A female, Comisana breed, a 6 month-old, clinically healthy sheep was included in the study as a control for the EEG patterns.

Table 1. EEGs in sheep with congenital hydrocephalus (cases 1 and 2), and in a control sheep (case 4): Relative power of the spectral bands (median values)

Case number	Delta (%)	Theta (%)	Alfa (%)	Beta (%)
Case 1	61.5	18	10	8
Case 2	74.5	6	8	9
Case 4	67.5	12.5	8	11

Electroencephalograms

Case 1. The EEG showed diffuse and synchronous, moderate to high-voltage slow-activity with superimposed high frequency activity (Tables 1 and 2, Figure 3A).

Case 2. The EEG showed low-voltage slow-activity with superimposed high frequency activity (Tables 1 and 2; Figure 3B).

Case 3. Due to technical problems, the EEG of this animal was not included in this study.

Case 4. The EEG showed moderate-voltage slow-activity with superimposed high frequency activity (Tables 1 and 2; Figure 3C).

The EEGs of all the examined sheep (except for case 3) were characterised by a considerable prevalence of slow activity.

Comparing the data of the three examined animals, in case 1 the prevalence of the theta activity was significantly higher compared to case 2 ($P < 0.000\,1$), and to case 4 ($P < 0.000\,1$). Furthermore, the prevalence of the theta activity in case 2 was significantly lower than in case 4 ($P = 0.019\,3$). The

Table 2. EEGs in ovis with congenital hydrocephalus (cases 1 and 2), and in a control ovis (case 4): Slow activity voltage

Voltage (μV)	Minimum	25% percentile	Median	75% percentile	Maximum
Case 1	15	33.25	45	66	118
Case 2	4	13	17	24	50
Case 4	16	27	33	40	64

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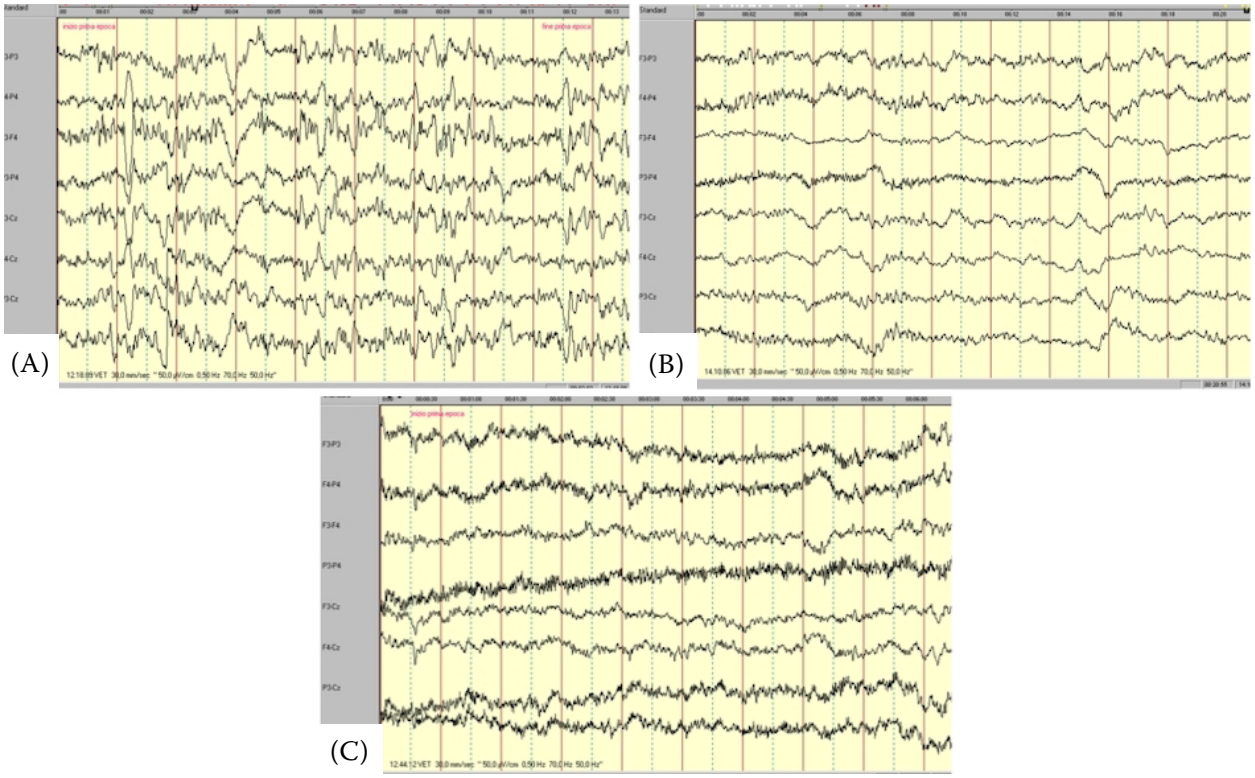

Figure 3. (A) EEG case 1: Diffuse and synchronous moderate-high voltage slow activity with superimposed high frequency activity. (B) EEG case 2: Low voltage slow activity with superimposed high frequency activity. (C) EEG case 4: Moderate voltage slow activity with superimposed high frequency activity

voltage of case 1 was significantly higher than case 2 ($P < 0.000\ 1$) and case 4 ($P < 0.000\ 1$), and the voltage of case 2 was also lower than case 4 ($P < 0.000\ 1$).

In case 2, the relative power of the delta activity was significantly higher in the frontal derivations (F3F4 vs P3P4: $P = 0.007\ 6$), while the relative power of the alpha band was significantly greater in the occipital area of the left hemisphere (P3Cz vs F3Cz: $P = 0.002\ 5$); on the other hand, the relative power of the beta activity was significantly higher in the occipital derivations (P3P4 vs F3F4: $P < 0.000\ 1$).

Anatomo-histopathological evaluation of the brains

Case 1. At necropsy, slight hyperaemia of the meningeal blood vessels, moderate pachygyria and dilatation of the ventricular system, particularly affecting the lateral ventricles, were observed (Figure 4A). Histologically, the wall of the ventricle was often irregular or absent and mild oedema of the periventricular white matter was detected (Figure 4B).

Case 2. Macroscopically, the inner surface of the cranial vault and its thickness were irregular. The brain was reduced in size, asymmetric, with rudimentary gyri, showing severe dilatation of the lateral ventricles (Figure 4C). Histologically, moderate, multifocal hyperplasia of the ependyma covering the lateral and third ventricles, and underlying multifocal, slight gliosis were present (Figure 4D). Moreover slight, multifocal non-suppurative choroiditis and slight, multifocal non-suppurative perivascular cuffs were detected in the neighbouring neuroparenchyma.

Case 3. At the post-mortem examination of the brain, moderate dilatation of the ventricles was detected. Histologically, moderate, multifocal hyperplasia of the lateral ventricles' ependyma was observed. Moreover, at the left cerebellopontine angle, a focal thickening of the meninges adherent to the neuroparenchyma and composed by different mesenchymal cells with elongated to epithelioid in shape, with abundant collagen was present. Multifocal moderate gliosis and non-suppurative perivascular cuffs were also detected in the neighbouring neuroparenchyma (Figure 4E–F).

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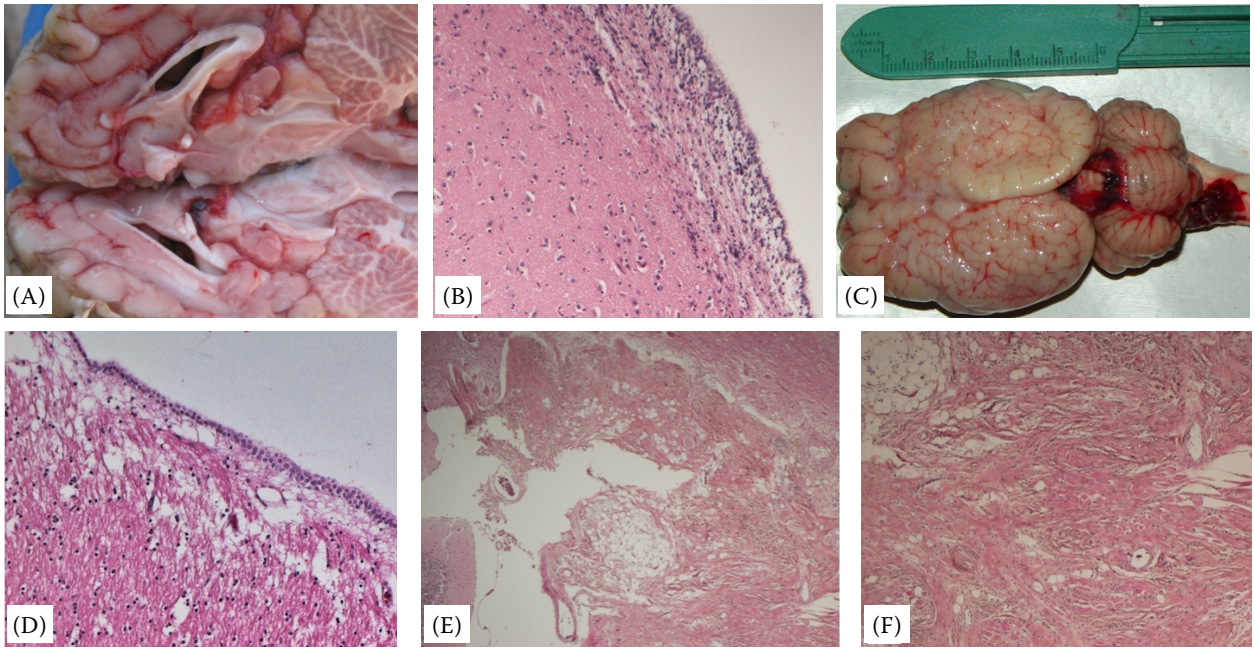

Figure 4. (A) Case 1: Moderate dilatation of the lateral ventricles. (B) Case 1: Multifocal slight hyperplasia of the ependyma, multifocal moderate gliosis and slight subventricular oedema. Haematoxylin and eosin (H&E), × 100. (C) Case 2: Collapse of the right cerebral hemisphere due to severe dilatation of the right lateral ventricle. (D) Case 2: Multifocal scattered hyperplasia of the ependyma and moderate subventricular oedema. Haematoxylin and eosin (H&E), × 100. (E) Case 3: Focal thickening of the meninges at the left cerebellopontine angle. Haematoxylin and eosin (H&E), × 25. (F) Case 3: Detail of the meningeal thickening composed of mesenchymal cells with variable pleomorphism arranged in bundles or small clusters, with abundant interstitial collagen. Haematoxylin and eosin (H&E), × 5

DISCUSSION

Congenital hydrocephalus in sheep is often incompatible with life, resulting in a high incidence of stillborn or perinatal mortality (Pritchard et al. 1994; de Lahunta and Glass 2009; Smith and George 2009). The affected animals described in the present work showed a long survival history, compatible with well-being till their slaughter or reaching adulthood, probably due to the moderate dilation of the ventricular system.

The observed neurological signs depend on the distribution and severity of the ventricle dilatation and the CSF compression. Alterations in the behaviour, mental status, postural and menace reactions are related to the damage in the telencephalon. On the other hand, when brain stem compression occurs, difficulty in sucking and swallowing, bruxism, fixed mydriasis, and postural reaction failure are present (Lavigne et al. 2003; Smith and George 2009). Clinical signs compatible with peripheral vestibular syndrome were detected in case 2. Enlargement of the calvarium, associated with fixed ventrolateral strabismus, was evident only in case 1.

Regarding the EEG pattern, the lack of standards in veterinary electroencephalography, concerning the state of the animal (sedated or awake), the type of sedation and positioning of the electrodes, gives rise to enormous variability between the studies and difficulties in comparing the results. Electroencephalographic records in clinically health sheep registered in wake and conscious states, showed different patterns depending on the age of the animals. The EEG in the first postnatal week shows a mixture of frequencies between 4 and 16 c/s and amplitudes reaching 30–50 μV (Pampiglione 1977). In one-year-old Polish Merino sheep, a bilaterally rhythmic activity with an amplitude ≤ 50 μV (mean 21 μV) was observed, corresponding to the theta rhythm (Cwynar et al. 2014). Channels located near the temporal line also detected delta wave activity and the activity recorded near the occipital lobes was characterised by a beta rhythm (Cwynar et al. 2014). In Polish Merino sheep aged 3–4 years, the EEG were characterised by a beta rhythm with a mean voltage value of 26.5 μV (Cwynar and Zawadzki 2010). There are two mechanisms producing EEG abnormalities in the hydrocephalus: the

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increased intracranial pressure that induces disturbances in the blood supply – either generally or focally – and cerebral oedema, and degenerative brain lesions secondary to the chronic increase of the intracranial pressure (Klemm and Hall 1971; Redding and Knecht 1984; Armasu et al. 2014). Changes in the EEG recording can differ between acute and chronic diseases: the rapid evolution of hydrocephalus may cause substantial brain dysfunctions which take longer to compensate for; while, when the evolution is chronic, the adaptative mechanisms allow the normal functioning of the brain and do not change the bioelectrical activity at the disease onset (Okurowska-Zawada et al. 2007).

The symmetric and synchronous high-voltage slow-activity, with a possible superimposed low-voltage fast-activity, described in dogs with hydrocephalus (Klemm and Hal 1971; Redding and Knech 1984; Armasu et al. 2014), was observed only in case 1.

All the sheep examined by EEG in the present work (two with hydrocephalus and one clinically healthy) had an EEG characterised by the prevalence of delta activity. However, in case 1, the voltage was significantly higher (median: 45 μV) than in cases 2 and 4. Also in literature, two studies reported lower voltage values in healthy sheep (Cwynar and Zawadzki 2010; Cwynar et al. 2014). Moreover, as reported by Cwynar et al. (2014), in case 2, the EEG activity varied depending on the area of the skull in which it was recorded: the beta rhythm had a greater expression in the posterior area while the delta rhythm had a greater expression in the most cranial zones. Cwynar et al. (2014) also reported the best locations for the EEG registration in the frontal, central and occipital regions, as undertaken in the present work.

Neuropathological investigations of the brain affected by congenital hydrocephalus do not always reveal macroscopical/histological features. If an evident loss of cerebral parenchyma or an obstruction of the CSF flow were not detected, the aetiology of the dilatation of the ventricles can remain unidentified. Sometimes, no abnormalities other than dilated ventricles can be observed, thus inferring that an alteration in the structure/function of the arachnoid villi, a developmental disorder at the subarachnoid level or an obstruction at the foramina of Magendie and Luschka along the medial and lateral walls of the fourth ventricle could be present. In this study, only one sheep (case 3) revealed a monolateral meningeal thickening at the left cerebellopontine angle that could be considered a potential cause of the limitation in the CSF circulation and of secondary obstructive hydrocephalus.

In the remaining two cases (cases 1 and 2), no obstructions in the CSF flow were detected, but focal chronic meningitis, subarachnoid haemorrhages/developmental disorders or alterations of the flow at the fourth ventricle level cannot be excluded. The brain of these sheep revealed a multifocal absence of the ventricular wall (case 1) and a mild reaction surrounding the dilated ventricles secondary to a chronic increase in the CSF pressure (oedema and glial reaction) (cases 1 and 2). These features are reported in the subependymal neuroparenchyma as a response to irritating non-specific insults, such as an imbalance in the cerebrospinal fluid pressure that occurred in the present cases (1 and 2) (Sarnat 1995). In case 2, the moderate hypoplasia of the cerebrum and the presence of multifocal non-suppurative choroiditis and non-suppurative perivascular cuffs does not exclude the possibility of an in utero viral infection producing inflammatory lesions and the consequent compensatory hydrocephalus, even if the biological agents were not investigated.

To conclude, the EEG patterns in sheep with hydrocephalus appear to undergo evident variations depending on the type and severity of the disease. However, multidisciplinary studies with a larger number of sheep could permit to better characterise the EEG pattern in ovine hydrocephalus.

Conflict of interest

The authors declare no conflict of interest.

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