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SHORT COMMUNICATION

Microcephaly and hydrocephalus in a sheep fetus infected with *Neospora caninum* in Southern Brazil – Short communication

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ABSTRACT

A case of non-communicating hydrocephalus and microcephaly in a sheep fetus infected with *Neospora caninum* from Lages, Santa Catarina, Brazil, is reported. Macroscopically, there was moderate flattening and narrowing of the skull, and the portion of the cerebral hemispheres was markedly reduced in size, measuring 3.5 × 3.5 × 0.5 cm, with marked diffuse flattening of the brain gyri and dilation of the lateral ventricles. Cerebrospinal fluid samples were positive to *N. caninum* detection by PCR. Histologically, there was discrete focal lymphoplasmacytic necrotising encephalitis on the floor of the lateral ventricle, discrete multifocal gliosis and discrete multifocal lymphoplasmacytic myositis. Through the molecular detection of *N. caninum* in the cerebrospinal fluid, it was possible to report what appears to be the first case of non-communicating hydrocephalus and microcephaly in an ovine fetus infected with *N. caninum*.

KEYWORDS

protozoan, anomaly, abortion, ruminants, encephalitis

Malformations in sheep fetuses and neonates can be attributed to many infectious agents, such as Schmallenberg virus (Dominguez et al., 2012), Border Disease, caused by a pestivirus (García-Pérez et al., 2009; Oguzoglu, 2012) and Bluetongue virus (Saegerman et al., 2011). Although hydrocephalus is a hallmark of congenital toxoplasmosis in humans, it is rare in livestock species and other animals (Dubey, 2022). *Neospora caninum* is widely reported as a cause of abortions in cattle (Dubey, 2003), with reports of this disease in sheep becoming increasingly frequent (Moreno et al., 2012; Dubey et al., 2017). The objective of this study is to report a case of non-communicant hydrocephalus and microcephaly in an ovine fetus infected with *N. caninum*.

A dead male Crioula sheep fetus from a farm located in Lages, Santa Catarina, Brazil, underwent necropsy. This lambing occurred without complications, being an isolated case of abortion on the farm. The sheep were raised extensively, supplemented with maize silage and sheep-specific mineral salt mixed with homoeopathic salt. No toxic plants that could be ingested by the animals were located in the pasture. There were four dogs on the farm with free access to the pastures, water sources and facilities, which sporadically ingested sheep fetal lochia afterbirths.

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During necropsy, the crown–rump length was compatible with 18–20 weeks of gestation (Sivachelvan et al., 1996). Externally, there was evidence of flattening and moderate narrowing of the skull cap. Opening the skull, it was observed that the telencephalon was reduced in size (Fig. 1A), and the portion of the cerebral hemispheres measured 3.5 × 3.5 × 0.5 cm, with marked flattening of the brain gyri (Fig. 1B). When cutting the brain, there was marked and diffuse dilation of the lateral ventricles, filled with cerebrospinal fluid (Fig. 1C). The weight of the brain mass was 12 g, consisting of 9 g of the telencephalic portion and 3 g of the cerebellum.

Fragments of all organs were stored in 10% buffered formalin and routinely processed for histopathological examination. There was evidence of discrete focal lymphoplasmacytic necrotising encephalitis, located on the floor of the lateral ventricles (Fig. 1D), as well as discrete multifocal gliosis and discrete multifocal lymphoplasmacytic myositis. For microbiological culture, fragments of liver, lung and abomasal content were collected, refrigerated and subjected to aerobic culture, without bacterial growth in the samples. Also, fragments of refrigerated liver were subjected to determination of hepatic copper concentration by flame atomic absorption spectroscopy. The hepatic copper concentration of the fetus was 68.8 mg kg^{-1}.

For molecular analysis, refrigerated brain and cerebrospinal fluid were submitted to a polymerase chain reaction (PCR) for *Toxoplasma gondii*, with primers SAG2.F4/SAG2.R4 (59GCTACCTCGAACAGGAACAC39)/(59GCATCAACAG TCTTCGTTGC39), amplifying a 340-bp product (Ossani et al., 2017) and *N. caninum*, using the primers Np21/Np6plus (5′-CCCAGTGCGTCCAATCCTGTA-3′)/(5′-CTCGCCAGT CAACCTACGTCTTCT-3′) amplifying a 337-bp product (Müller et al., 1996). Refrigerated spleen and thymus were submitted to RT-PCR for the pestivirus that causes Border Disease, with primers PanPesti F/PanPesti R (5′-GAGATGC YAYGTGGACGAGGG C-3′)/(5′-GYCTCTGCSRCACCCTA TCAGG-3′) amplifying a product of 118 bp of the 5′ untranslated region (5′ UTR) of ruminant pestiviruses (Weber et al., 2014). Also, refrigerated spleen, thymus and liver were submitted to RT-PCR for bluetongue virus, with the modified primers BTV IVI F2/BTV IVI F2 (5′-TGGAYAAAGCRA TGTCAAA-3′)/(5′-ACRTCATCACGAAAGGCTTC-3′) for the region Seg-10 (Van Rijn and Boonstra, 2021). The results for the detection of *T. gondii*, ruminant pestivirus and bluetongue were negative, while the cerebrospinal fluid was positive for *N. caninum* detection. All positive controls consisted of already known positive cases for each infectious agent, while the negative control consisted of autoclaved ultrapure water.

Hydrocephalus is a commonly observed congenital abnormality in domestic animals (Schmidt and Ondreka, 2019). In this fetus, it can be classified as non-communicating, visualised in obstructive or stenotic processes, secondary to infectious, neoplastic or degenerative causes, resulting in dilatation of the ventricles before flow

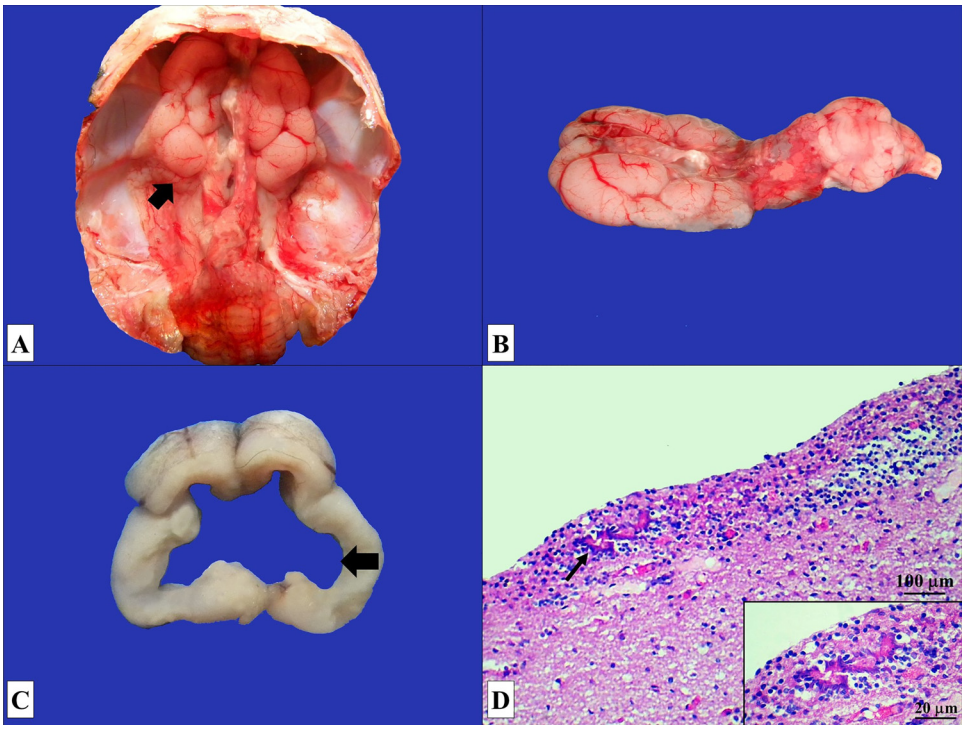

Fig. 1. Sheep fetus with microcephaly and hydrocephalus secondary to *Neospora caninum* infection. **A:** Sharp size decrease in the telencephalon (arrow). **B:** Lateral view of the brain, showing marked flattening of the brain gyri and telencephalic mass. **C:** Sharp dilatation of the lateral ventricles (arrow). **D:** Brain: discrete focal lymphoplasmacytic necrotising encephalitis, located in the cerebral cortex, at the level of the floor of the lateral ventricles (arrow). Magnification: ×10. In detail: a zoomed-in image of the injury. Magnification: ×40. Haematoxylin and eosin

obstruction (Cantile and Youssef, 2016). Among the non-infectious causes of malformations, copper deficiency in sheep can lead to congenital alteration known as enzootic ataxia, resulting from demyelination, which usually occurs as outbreaks, with liver copper values of about 4–6 mg kg^{-1} (Sousa et al., 2012), far below the value obtained in this fetus, ruling out copper deficiency as one of the possible differentials.

Viral causes of malformations, such as pestiviruses and bluetongue (Saegerman et al., 2011; Oguzoglu, 2012), usually culminate in porencephaly, hydranencephaly, hydrocephalus and cerebellar hypoplasia in ovine fetuses, constituting two important differentials for the macroscopic changes evidenced in this case, discarded after the negative detection results of fetal tissues. The pathogenesis of hydrocephalus associated with protozoa may be related to inflammatory damage in the subependymal tissue near the ventricles, with cell desquamation in the ventricles, resulting in blockage and dilation of the lateral ventricles, as previously reported in cases of toxoplasmosis in humans (Hutson et al., 2015). The evidence of foci of lymphoplasmacytic necrotising encephalitis located on the floor of the lateral ventricles reinforces the hypothesis that in this fetus, the emergence of hydrocephalus may have followed these events.

Neosporosis is gaining increasing notoriety as a cause of mortality in fetuses of small ruminants, becoming increasingly common (Moreno et al., 2012), however, malformations associated with *N. caninum* are rare, and never had been reported in sheep. In cattle, there was a case of *ex-vacuo* hydrocephalus in an aborted fetus infected by this protozoan (Dubey et al., 1998). In goats, it has been reported in a fetus with hydrocephalus and cerebellar hypoplasia (Dubey et al., 1996). These cases of *ex-vacuo* hydrocephalus had foci of brain necrosis with greater distribution and intensity, suggesting that the most compatible pathogenesis for this case is a flow obstruction of the cerebrospinal fluid, and not a compensatory expansion of the ventricles due to malacia in the brain tissue.

PCR presents high sensitivity and specificity for the diagnosis of neosporosis (Dubey, 2003). The absence of *N. caninum* detection in brain tissue, in this case, and the presence of its DNA in the cerebrospinal fluid, supports that due to the scarcity of central nervous system tissue and the location of inflammatory lesions close to the ventricular floor, the cell desquamation in the ventricular space was favoured.

Microcephaly is mostly seen in the the cerebral hemispheres with a reduction in equal proportions of the white and grey matters, giving the impression of an unusually large cerebellum. In cases of microcephaly, it is possible to externally identify a flattening and narrowing of the skull, as seen in this sheep fetus (Vandevelde et al., 2012; Cantile and Youssef, 2016). The weight ratio of the cerebellum to the brain of normal animals can vary from 8.3 to 11.01% (Done et al., 1980), lower than in this case, in which the cerebellum was equivalent to 25%, corroborating the findings that microcephaly leads to more significant reductions only in the cerebral hemispheres.

Microcephaly arises from defects in neurogenesis associated with disruption of the proliferation of neural progenitors through pathogens that can lead to defects in the mitotic division or regulation of their cell cycle, or also from defects resulting from DNA damage, leading to a reduced number of neuronal and glial cells in the brain (Passemard et al., 2013). Protozoal infection leading to microcephaly remains with obscure pathogenesis, even for humans with congenital infection by *T. gondii* (Devakumar et al., 2018), and in the present case, the marked reduction in the proportions of cerebral hemispheres may be associated with inflammatory lesions localised in the subventricular zone, culminating in the destruction of neuronal progenitors. To date, no cases of microcephaly associated with *N. caninum* in domestic animals have been reported.

This appears to be the first report of hydrocephalus and microcephaly in a sheep fetus infected with *N. caninum* in Southern Brazil, with molecular detection of the protozoan in the cerebrospinal fluid. The diagnosis was based on PCR and histopathology, and the exclusion of other infectious causes supported the diagnosis. Although never before reported as a teratogenic agent in sheep, the occurrence of *N. caninum* in this case alerts to its potential as a cause of malformations in this species. As such, and along with other infectious agents, it should be considered as a differential for this type of alteration in ovine fetuses and newborn sheep.

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