Arthrogryposis Multiplex Congenita due to Toxoplasma gondii Infection in a Newborn Calf
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Abstract
Arthrogryposis Multiplex Congenita (AMC) includes various conditions of unclear etiology but in general any cause of reduced fetal movement may result in congenital contractures. Neurologic abnormalities appear as the most common causes of AMC (approximately 70% to 80% of all cases). This paper describes a case of clinical congenital toxoplasmosis in a newborn calf and suggests that the protozoan Toxoplasma gondii may also be responsible for AMC. In fact, a central nervous system involvement appears to be present in most cases of AMC and in this case the presence of Toxoplasma gondii in calf’s brain was demonstrated through molecular biology examination and immunohistochemistry analysis.

Keywords: Arthrogryposis; Congenital toxoplasmosis; Cattle; Real time PCR; Immunohistochemistry

Materials and Methods
Samples for histopathology analysis (central nervous systems, lung, liver, spleen, intestine, muscle, heart and kidney) were fixed in 10% neutral buffered formalin, processed routinely, sectioned at 2-3 μm and stained with hematoxylin and eosin. Same samples were performed for immunohistochemical examination (IHC) for the detection of Toxoplasma gondii. IHC was performed using the EnVision+Dual Link System-HRP (Dako, Denmark). This system is a two-step IHC staining technique based on an HRP labelled polymer, which is conjugated with secondary antibodies. The labelled polymer does not contain avidin or biotin. Consequently, non-specific staining resulting from endogenous avidin-biotin activity is eliminated or significantly reduced. Any endogenous peroxidase activity was quenched by incubating the tissues for 5-10 minutes with Dual Endogenous Enzyme Block. The tissues were then incubated with a ready-to-use primary antibody versus Toxoplasma gondii (rabbit polyclonal, Thermo Fisher ScientificUK), followed by incubation with the labelled polymer using a 30 minute incubation for each. Staining was completed by a 5-10 minutes incubation with 3,3'-diaminobenzidine (DAB+) substrate-chromogen which results in a brown-colored precipitate at the antigen site. Heart, spleen and brain samples were examined for Toxoplasma gondii by AF targeted real time PCR. The spleen was also examined for Neospora caninum by PCR. Other lung, liver, spleen, intestine, heart, brain and kidney samples were examined for Adenovirus (AV) by tissue culture, for Bovine Herpes virus 4 (BHV4) by tissue culture, for Bovine Viral Diarrhoea virus (BVDV) by tissue culture and antigen capture Enzyme-Linked Immunoabsent Assay (ELISA), for Schmallenberg virus (SBV) by real time PCR, for Infectious Bovine Rhinotracheitis (IBR) by tissue culture, for Bovine Arthropodborne Encephalitis (BACE) by tissue culture, for Bovine Respiratory Syncytial virus (BRSV) by tissue culture and for Bluetooge virus (BTV) by tissue culture.

Results and Discussion
Observed lesions were flexure of the carpal and tarsal joints and flexure of the metacarpophalangeal and metatarsophalangeal joints in combination with a moderate lateral rotation of the phalanges causing medial deviation of the fingertips. No limb bone, joint or muscle lesions were found. No others skeletal malformations were apparent. The head of the stillborn calf was normal in size. No secondary palatoschisis, brachygnathia superior or tongue protrusion were found. Congenital toxoplasmosis is rarely reported in cattle despite the possibility of reproductive disorders, such as abortion, being due to the infection. In Switzerland, Gottstein et al. detected Toxoplasma gondii DNA in 5% of bovine fetuses with viable Toxoplasma gondii isolated in two aborted fetuses. It seems clear that Toxoplasma gondii can be transplacentally transmitted in cattle, but that this is probably not a common occurrence. Furthermore, there is very little evidence for the correlation between AMC and toxoplasmosis in cattle.

Conclusion
A central nervous system involvement appears to be present in most cases of AMC and in this case the presence of Toxoplasma gondii in calf’s brain was demonstrated through molecular biology examination and IHC. In affected calf, infection with Toxoplasma gondii had resulted in a microglial cell response at various stages of activation with formation of nodules. This response was not associated with any other evidence of acute inflammation and the only histopathological manifestation of Toxoplasmic encephalitis was the presence of microglial nodules scattered through the brain. No vascular proliferation, endothelial hyperplasia, perivascular inflammatory infiltrate or necrosis was found. The brain lesion due to congenital toxoplasmosis infection can therefore be considered the cause of the multiple joint contractures and consequent AMC. This paper demonstrates how congenital toxoplasmosis, in addition to wellknown fetal abnormalities (hydrocephalus, intracranial calcification, choriotiretinitis, etc.) may also be responsible for AMC.

References

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