J. Comp. Path. 2014, Vol. 151, 148–152

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INFECTIOUS DISEASE

Skeletal Muscle Hypoplasia Represents the Only Significant Lesion in Peripheral Organs of Ruminants Infected with Schmallenberg Virus during Gestation

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Summary

Schmallenberg virus (SBV), an arbovirus within the family Bunyaviridae, represents a ruminant pathogen that has caused epidemic abortion and birth of malformed or stillborn animals in many European countries since August 2011. Histological and immunohistochemical analysis of peripheral tissues of SBV-infected animals, including lymphoid tissues, endocrine organs and tissues of the gastrointestinal, urogenital and respiratory system, were analyzed in order to elucidate the occurrence of SBV-associated changes and the presence of viral antigens and RNA. Twenty calves and 12 lambs as well as age-matched controls were included in this study. Significant muscular hypoplasia with fatty replacement was noted in affected calves and lambs. In addition, hepatocellular degeneration with lymphohistiocytic inflammation, interstitial fibrosis and biliary hyperplasia was detected in calves. All animals lacked SBV-positive cells in the peripheral organs. These observations resemble those found in Akabane virus- and Cache Valley virus-infected animals and support the occurrence of few residual lesions in peripheral organs following SBV infection.

Keywords: calf; lamb; muscle hypoplasia; Schmallenberg virus

In autumn 2011, a new orthobunyavirus within the family Bunyaviridae emerged in Germany and neighbouring countries and was termed Schmallenberg virus (SBV; Hoffmann *et al.*, 2012). This ruminant pathogen caused fever, transient decrease in milk yield and diarrhoea in adult cattle as well as epidemic abortion, malformation and perinatal death of calves, lambs and goat kids. Typical macroscopic findings of SBV infection include brachygnathia inferior, torticollis, kyphosis, lordosis, scoliosis and arthrogryposis. In addition, pathological findings in the central nervous system (CNS), such as cerebellar and cerebral hypoplasia, hydranencephaly, porencephaly, hydrocephalus and micromyelia, are frequently present (Herder *et al.*, 2012). CNS lesions are related to SBV infection of neurons, which is followed by perivascular lymphohistiocytic encephalitis, especially in the mesencephalon as well as the temporal and parietal lobes (Hahn *et al.*, 2013; Herder *et al.*, 2013; Varela *et al.*, 2013). Macroscopic and histological findings are similar to changes described in aborted or newborn ruminants infected with Akabane virus (AKAV), Aino or Cache Valley virus (Konno *et al.*, 1982; Tsuda *et al.*, 2004; Rodrigues Hoffmann *et al.*, 2012). Hypoplastic muscular changes are a characteristic finding in lambs and calves after natural or experimental infections with Orthobunyaviruses, as well as in hamsters and chicken embryos infected experimentally with AKAV. However, no lesions are found in any other visceral organs and tissues (Saito

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.04.006

et al., 1981; Konno *et al.*, 1982, 1988). The aims of this study were to characterize the pathological changes occurring in the peripheral organs of SBV-infected, neonatal or stillborn lambs and calves and to determine whether SBV antigens or RNA are present in these organs.

Samples were collected from the peripheral organs of SBV-infected ruminants from Germany. The animals selected were confirmed as having SBV infection by polymerase chain reaction (PCR) testing of brain tissue and meconium as previously described (Herder *et al.*, 2012). Twenty calves and 12 lambs were used in this study. Samples were fixed in formalin and embedded in paraffin wax (Hahn *et al.*, 2013; Herder *et al.*, 2013). All available organ systems were investigated including skeletal muscles, heart, peripheral nerves, skin, eyes, lymphoid tissues and gastrointestinal, respiratory and urogenital tracts as well as endocrine organs. Skeletal muscle tissue investigated was derived from the pectoral girdle, the proximal region of the forelimbs and the thigh.

Age-matched controls consisted of 30 calves and seven lambs. Tissues from these animals were retrieved from the archive of the Department of Pathology, University of Veterinary Medicine, Hannover. Although these animals were not investigated for SBV infection, they were submitted for necropsy examination prior to the emergence of SBV (Gerhauser *et al.*, 2014). All control tissues were either from aborted animals (with and without malformations) or from neonates. Statistical analysis of the occurrence of pathological changes between the SBV-infected group and controls was made by a chi square test, with $P \leq 0.05$ taken as significant.

Sections (2–3 μm) were stained with haematoxylin and eosin (HE). Selected tissue samples from the liver were stained with azan for detection of fibrosis. Immunohistochemistry (IHC) for SBV antigen was performed as described previously (Herder *et al.*, 2013). Immunohistochemical labelling for desmin was performed on selected skeletal muscles from SBV-infected animals and controls. A polyclonal rabbit antibody directed against the SBV N protein diluted 1 in 3,000 (Varela *et al.*, 2013) and a primary monoclonal mouse antibody against desmin diluted 1 in 100 (Dako, Hamburg, Germany) were used. Negative control sections were incubated with normal rabbit serum (SBV) or with an isotype-matched control antibody (desmin). In-situ hybridization (ISH) to detect SBV mRNA was performed on selected sections including tissues displaying lesions as described by Hahn *et al.* (2013).

Five out of 12 SBV-infected lambs (41.7%) and 16 of 20 (80%) infected calves showed moderate to marked muscular hypoplasia with replacement of myocytes by adipose tissue and multifocal mild fibrosis. The few remaining muscle fibres were thin and small (Fig. 1). The cross-striation was lost in some of the muscle fibres and replaced by cytoplasmic hypereosinophilia. Nuclei of the fibres were spherical to cigar shaped and were located at the cell periphery. Inflammatory infiltrates were not found in any muscle tissue. Control calves and lambs showed no muscular changes. Statistical analysis confirmed that muscular hypoplasia occurred more frequently in SBV-infected lambs and calves compared with controls ($P < 0.0001$ for calves; $P = 0.037$ for lambs).

Mild to moderate fibrosis with biliary hyperplasia and mild to moderate, multifocal, periportal lymphohistiocytic hepatitis were seen in seven out of 20 SBV-infected calves (35%). Similar changes were present in the liver of five of 30 control calves (17%), but the difference was not significant ($P = 0.11$).

Two SBV-infected lambs showed mild to moderate, multifocal necrosuppurative hepatitis. One ovine control had mild lymphohistiocytic hepatic infiltrates and mild biliary hyperplasia was present in one control lamb ($P = 0.865$).

SBV-infected lambs and calves showed fetal atelectasis, sometimes with mild aspiration of meconium. Additionally, mild alveolar histiocytosis and, in one case, mild infiltration with neutrophils was found. Occasionally, mild lymphoid depletion was detected in the spleen and thymus, as well as in lymph nodes of some animals. Two animals showed a mild to moderate infiltration of neutrophils and extramedullary haemopoiesis in the spleen. The eye of one lamb was affected by mild lymphohistiocytic to suppurative keratitis. The intestinal tract of three calves showed mild infiltration of eosinophils and a few crypt abscesses. Mild infiltration of neutrophils, lymphocytes and macrophages was found in an adrenal gland of one lamb and one calf.

Heart, kidneys, testes/ovaries, uterus, placenta, peripheral nerves, thyroid glands, pituitary gland, umbilical cord and skin were without significant microscopical changes in SBV-infected calves and lambs.

Control animals displayed few lesions. One control lamb had mild suppurative pneumonia, one had mild splenic lymphoid depletion, one had mild lymphoplasmacytic tracheitis and one had cysts in the thyroid gland. Five control calves had fibrinosuppurative pneumonia of varying degrees. Lymph nodes and/or spleen of four control calves had lymphoid depletion. Six control calves had mild to moderate purulent inflammation of the navel, spleen, cornea and lymph node. No significant differences were