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Experimental bluetongue virus serotype 8 infection of Swiss sheep: Special emphasis on clinicopathological picture, virological and diagnostical features and the transplacental transmission of the virus

Gabriella Worwa

Bluetongue (BT) is an arboviral, hemorrhagic disease of ruminants which is caused by bluetongue virus (BTV) of the family *Reoviridae*. There are at least 24 different serotypes of BTV that are endemic in geographical areas between latitude 40°S and 53°N in Africa, Middle East, Australia, Asia and America. Prior to 2006 BT never appeared in northern parts of Europe including Switzerland and therefore was considered as an emerging disease. However, in autumn 2007 BTV serotype 8 (BTV-8) emerged in Switzerland for the first time and released a still ongoing epidemic having serious impact on Swiss livestock.

Female midges of *Culicoides spp*. are the biological vector of BTV and transmit the virus on susceptible hosts by bites. A broad spectrum of domestic and wild ruminant species is susceptible to BTV infection, but sheep and certain deer species in particular are regarded as hosts showing the most pronounced disease manifestation. As sheep are the most sensitive species in terms of BT surveillance based on clinical disease, this study aimed to assess the pathogenicity of BTV-8 for indigenous sheep breeds. Therefore, sheep belonging to the four most common Swiss breeds (Swiss Black-brown Mountain sheep, Brown-headed Meat sheep, Swiss White Alpine sheep, Valais Black Nose sheep) and sheep of a highly susceptible English breed (Poll Dorset sheep) were experimentally infected with the northern European strain of BTV-8.

Infected animals of all breeds developed BT-typical clinical signs such as pyrexia, apathy, congestion, edema, hyperemia and hemorrhages of mucous membranes and skin, oral erosions and ulcers, respiratory disorders like serous to mucous nasal discharge and, in cases of fulminant BT, acute lung edema and respiratory distress.

Necropsy performed at acute stages of infection revealed BT-typical pathology including hemorrhages, effusions, edema, erosions and activation of lymphatic tissues. Hemorrhages on the base of the *arteria pulmonalis* and the left *musculus papillaris subauricularis* were frequently present. Histology confirmed the macroscopical findings.

In pregnant, BTV-8-infected ewes a transplacental infection of the fetus was observed, which is the first report on intrauterine transmission of northern European BTV-8 in sheep and represents a unique feature of wild-type BTV-8.

Viremia was assessed in all animals by performing real-time reverse transcriptase PCR (rRT-PCR) using viral ribonucleic acid (RNA) from time course EDTA blood samples. All infected sheep became viremic between 3 and 7 days *post infectionem* (d.p.i.) and remained positive until the end of the trial. In three breeds long-term BTV-8 viremia was demonstrated up to 133 d.p.i. Different tissues were analyzed regarding virus load by detection of viral RNA and infectivity by cell culture isolation. Spleen and lung were the organs with the highest amount of BTV-8 and viral RNA could be detected in certain tissues even after 151 d.p.i. Virus detection by rRT-PCR testing revealed a high sensitivity, whereas cell culture isolation did not represent a reliable way of BTV detection.

Using statistical comparisons and novel score systems, a correlation between clinical manifestation and pathology was found to be significantly related. Furthermore, clinical signs and fever were shown to be indicative for the concurrent presence of high amounts of viral RNA in blood.

Conclusively, animals of all breeds developed clinical disease, viremia and pathological lesions, demonstrating that BTV-8 is fully capable of replicating and inducing BT in the investigated Swiss sheep. No significant breed-related differences were observed and therefore it can be concluded that the Swiss sheep are as susceptible to BTV-8 infection as the highly susceptible English Poll Dorset sheep. Moreover, the reproduction of fulminant BT in this study emphasizes a remarkably high virulence of BTV-8 for indigenous sheep.