

An outbreak of sheep-associated malignant catarrhal fever in sows

Een gediagnosticeerd geval van schaapgeassocieerde boosaardige catarraal koorts bij zeugen

¹B. Mateusen, ²P. Vyt, ¹S. Ribbens, ³S. Van Colen, ⁴C. Letellier, ⁴P. Kerkhofs, ⁵H. Nauwynck, ¹D. Maes

¹Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

²Medic Lab, Diagnostic Laboratory, Zonnestraat 3, 9300 Aalst, Belgium

³Aveve Animal Nutrition, Eugeen Meeusstraat 6, B-2170 Merksem, Belgium

⁴Veterinary and Agrochemical Research Center, Department of Virology, Groeselenberg 99, B-1180 Brussels, Belgium

⁵Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

Dominiek.Maes@UGent.be

ABSTRACT

This paper describes a case of malignant catarrhal fever in a sow herd in Belgium caused by infection with ovine herpesvirus-2 (OHV-2). The 11 affected sows had high fever and 10 of them died within 3 days after the onset of clinical disease. The most prominent macroscopic lesion was a hemorrhagic to pseudo-membranous gastritis. Histopathology revealed severe infiltration and necrosis of the gastric mucosa. Neither antimicrobial treatment nor injection with anti-inflammatory drugs ameliorated the severity of the disease. As the sows and sheep were housed in the same building with the possibility of having direct nose-to-nose contact, and as PCR testing showed that the virus found in the sows was identical to that found in the sheep, it is very likely that the infection was transmitted from the subclinically infected sheep to the sows. The present case showed that OHV-2 infection should be included in the differential diagnosis when facing problems of fever followed by death, especially when pigs are housed in close contact with sheep.

SAMENVATTING

In dit artikel wordt een geval van boosaardige catarraal koorts beschreven in een zeugenbedrijf in België. De koorts werd veroorzaakt door een infectie met het ovine herpesvirus-2 (OHV-2). De 11 aangetaste zeugen hadden hoge koorts en 10 ervan stierven binnen de 3 dagen na het verschijnen van de klinische symptomen. Het meest uitgesproken macroscopische letsel was een hemorragische tot pseudo-membraneuze gastritis. Het histopathologische onderzoek toonde een erge infiltratie en necrose van de maagmucosa aan. Noch een behandeling met antibiotica noch een injectie met anti-inflammatoire middelen kon de ergheid van de ziekte verminderen. Aangezien de zeugen in dezelfde ruimte gehuisvest waren als de schapen, met de mogelijkheid tot direct neus-tot-neuscontact, en aangezien de PCR-resultaten aantoonde dat het virus bij de zeugen en de schapen identiek was, is het zeer waarschijnlijk dat de infectie werd verspreid van de subklinisch geïnfecteerde schapen naar de zeugen. Deze uitbraak toont aan dat OHV-2 moet opgenomen worden in de differentiaaldiagnose indien er koorts optreedt gevolgd door sterfte, vooral wanneer varkens nauw contact hebben met schapen.

INTRODUCTION

Malignant catarrhal fever (MCF) has been recognized as a significant emerging problem for several ruminant species worldwide (Ackermann, 2006). The causative agent of this disease, which is mainly fatal in cattle (O'Toole *et al.*, 1997), can have a dual origin. One disease form takes place with sheep as transmit-

ting carrier species and has been characterized as an ovine herpesvirus-2 (OHV-2) infection (Baxter *et al.*, 1993). The other form has been associated with the wildebeest (*Connochaetes* species) and is characterized as the alcelaphine herpesvirus-1 (AIHV-1) infection (Plowright *et al.*, 1960). Both viruses are members of the *Gammaherpesviridae*, genus *rhadinovirus* (Li *et al.*, 2005; Ackermann, 2006).

Besides the well described clinical disease form in ruminants such as domestic cattle (Liggitt and DeMartini, 1980a,b; Deprez *et al.*, 2004), buffaloes (Tham, 1997), bison (Li *et al.*, 2006) and cervids (Li *et al.*, 2003, Keel *et al.*, 2003), other animal species are also susceptible to infection with this viral agent. In the literature, porcine malignant catarrhal fever has been described either as suspected cases based on the presence of clinical symptoms (Holmgren *et al.*, 1983, Okkenhaug and Kjølvik, 1995), or, more recently, as diagnosed cases in Norway and Switzerland, through the use of newly developed polymerase chain reaction (PCR) assays (Loken *et al.*, 1998, Albini *et al.*, 2003). In these latter cases, limited outbreaks of MCF caused by OvHV-2 were diagnosed. The disease outbreaks in pigs have always been associated with non-clinical infection in sheep and the presence of sheep nearby pigs. The rather sporadic occurrence of the disease in porcine hosts has led to the fact that MCF is seldom included in differential diagnoses when clinical problems are encountered in pigs. In addition, the true incidence of MCF in pigs is probably also underestimated because appropriate diagnostic techniques were not available in the past (O'Toole *et al.*, 1997).

This paper describes a severe outbreak of MCF in sows in a Belgian farrow-to-finish pig herd.

MATERIALS AND METHODS

Herd description and farm management

The affected herd was a farrow-to-finish pig herd of 110 crossbred sows (Belgian landrace x English or Finnish landrace or York). The pregnant sows were housed individually in crates and received approximately 2.5 kg per day of a commercial dry meal throughout gestation. Seven days before the expected farrowing date, the sows were moved to the farrowing unit, in which they were housed in individual farrowing crates. After farrowing, the sows were given a commercial lactation feed, which was provided *ad libitum* from 7 days post-farrowing onwards. The drinking water, originating from a deep pit located on the farm, was provided *ad libitum* for sows in all reproductive stages.

Sheep were also present on the farm. From November 2005 till March 2006, 4 adolescent sheep that were approximately 8 months of age (November 2005) were housed in the same barn as the pregnant sows. They were kept in a pen immediately adjacent to the pregnant sows, separated only by a fence of slatted iron bars. In this way, adolescent sheep had direct nose-to-nose contact with the neighboring sow and with sows that escaped from their individual crates. Starting from 15 February 2006, sheep that were 1 week before expected parturition date were moved to the sow farrowing units and were housed next to sows that either had already farrowed or were in their last week of gestation. The sheep were kept in the sow farrowing units until 3 weeks after lambing. There, as well, direct nose-to-nose contact between sheep and sows was possible because they were separated only by a wooden board 40cm high.

Case history

On 27 January 2006, an eight-week pregnant sow suddenly died without any preceding indications of illness. Four days later, another sow that was 6 weeks pregnant became anorectic and showed signs of depression. The rectal temperature was 40.7°C and the respiration rate was high. Despite parenteral treatment with non-steroid anti-inflammatory drugs and antibiotics, her condition got worse. Before dying on the second day after disease onset, the sow developed other clinical features such as hemorrhagic skin lesions, muscular tremor, convulsive movements and hyperemia of the conjunctivae. On 11 February, 4 sows that were between 3 and 8 weeks pregnant displayed clinical symptoms similar to those mentioned above. Three of them died within 3 days. In the week of 23 February, another 5 sows died after developing similar clinical symptoms. Two of these sows were housed in the farrowing unit and the other 4 were housed in the gestation unit and had been inseminated approximately 3 weeks before. During this disease outbreak, a total of 11 sows got severely ill, with 10 of them dying within 3 days after onset of disease. In one case, abortion was noted prior to death. One sow survived after a week of anorexia and remained a poor-doer, displaying a severe loss of body condition.

Pathology and histopathology

Post-mortem examination of six of the 10 dead sows and one litter of aborted fetuses was performed at the regional diagnostic laboratory. Three dead sows were examined on-farm, and from 1 of these sows, the stomach was sent to the regional laboratory (Animal Health Care Flanders, Torhout, Belgium) for further investigations. The first dead sow was not presented for necropsy. At the laboratory, necropsy was performed and samples for bacteriological and histological examination were taken. The latter samples were fixed in 4% buffered formalin, embedded in paraffin and stained with hematoxylin-eosin. Additionally, tissue with lesions was stored at -20°C for further examination.

Microbiological and serological assays

Organs of necropsied sows were inoculated on Columbia agar with sheep blood (Oxoid, Drogen, Belgium) and MacConkey agar number 3 (Oxoid, Drogen, Belgium) for standard bacteriological examination. In addition, the organs were inoculated on campylobacter CCDA selective medium, BPLS agar and Yersinia selective medium (Oxoid, Drogen, Belgium) to detect *Campylobacter*, *Salmonella* and *Yersinia*. Isolation of *Brachyspira* species was attempted using homemade media (Råsbäck *et al.*, 2005). Bacteriological examination was also done on blood samples from two sows in the acute phase of the disease, before treatment. Bacteriological examination of the drinking water was performed using plate count

agar (Oxoid, Drogen, Belgium) to determine colony forming units.

Tissue samples from 2 stomachs were examined using a PCR assay specific for the OHV-2, as described by Baxter *et al.* (1993). Blood samples were taken from sows that came in close contact with the sheep, including sows of different ages and both normal and suspected sows. Blood samples were also taken from the sheep. Blood samples from sows and sheep were examined for OHV-2 using a PCR assay (Baxter *et al.*, 1993).

RESULTS

Pathology

The most prominent macroscopic lesion on gross post-mortem examination in all 6 sows was a hemorrhagic to pseudo-membranous gastritis (Figure 1). Histology revealed a severe vasculitis and necrosis of gastric mucosa. Additionally, fibrinous enteritis was seen in the small intestine of one sow and in the colon of another. Hemorrhagic, circular skin lesions ranging from 2 to 20 mm diameter were seen in two sows, especially on the hind limbs, udder and neck (Figure 2). Histologically, there was a severe vasculitis in the skin. Severe congestion of the spleen was noted twice, and in one sow, several pale spots with a diameter of 2 mm were seen on the kidneys. These pale spots were characterized histologically as an interstitial infiltration of mononuclear cells. Histopathological examination of the brain revealed a high-grade meningo-encephalitis in all three cases examined (Figure 3). Infiltration of mononuclear cells was most prominent around small blood vessels and in the meningeal space.

Microbiological and serological assays

Standard bacteriological examination of the different tissue samples did not reveal any specific pathogens that could account for the observed lesions. Cultures for *Salmonella* sp., *Yersinia* sp. and *Brachyspira* sp. were negative. No bacteria could be isolated from the blood samples of the two clinically diseased sows. The results of the bacteriological examination of the drinking water were as follows: 120 cfu/ml at 37°C, 98 cfu/100ml for fecal *Streptococci*, and 30 cfu/20ml sulphite reducing *Clostridia*.

The PCR testing for OHV-2 applied to stomach samples from 2 affected sows was positive (Figure 4, lanes 1 and 2). Next, blood samples from a negative control (lane 3), 4 sows (lanes 4 to 7), 2 aborted fetuses (lanes 8 and 9) and 3 contact sheep (lanes 10 to 12) were analyzed using the same PCR test. The presence of OHV-2 DNA was detected in all sheep. OHV-2 DNA was also detected in the blood of the surviving sow 6 weeks after its clinical illness (lane 6). A second nested PCR was performed on the negative samples, as described in Baxter *et al.* (2001). All tested samples remained negative (data not shown). The PCR products



Figure 1. Hemorrhagic to pseudo-membranous gastritis in a sow that died of OHV-2 Infection.



Figure 2. Circular hemorrhagic skin lesions in a sow that died of OHV-2 infection.

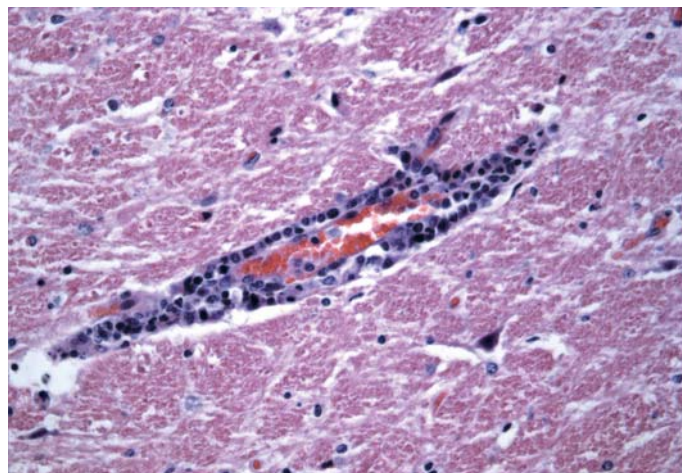


Figure 3. Histopathology of the brain stem (HE staining 400x): non-purulent meningo-encephalitis.

obtained from the positive sows and from the 3 sheep were purified and then subjected to sequencing analysis, with the sequences aligned. A 100% similarity was observed between the sequences of the sow virus and the sheep virus (Figure 1). The similarity with a published OHV-2 tegument sequence was 99.7% (Genbank accession number L05908), thus demonstrating that the animals were infected with OHV-2.

DISCUSSION

The sows in the present herd were diagnosed to be infected with MCF. Based on the disease history, the anamnesis, the clinical symptoms and the PCR-positive results in the sheep, it is very likely that transmission of OHV-2 from the sheep to the sows was responsible for the MCF outbreak.

In susceptible ruminant species, the incubation period for developing MCF using experimental exposure varies considerably, namely from 9 to over 60 days (Plowright *et al.*, 1960; Stadtfeld and Haberkorn, 1986). Information regarding the incubation period for developing MCF in pigs is not available. In the present case, the sows got ill in a period between 68 and 92 days after adolescent sheep were moved immediately adjacent to susceptible sows. This time period is comparable to that of an outbreak of MCF in a bison feedlot in which peak losses occurred between 41 and 55 days post-exposure (Li *et al.*, 2006).

In comparison to the other presumed or diagnosed cases of porcine MCF (Holmgren *et al.*, 1983; Okkenhaug and Kjellvik, 1995; Loken *et al.*, 1998; Albini *et al.*, 2003), the present outbreak was more severe, with a total of 11 sows (or 9% of the sow herd inventory) being affected. In general, the extent of a MCF outbreak is determined by the susceptibility of the species, the virus load shed by carrier animals, and the intensity of contact between the carrier animals and the susceptible species. Whether the rather low prevalence of reported MCF cases in pigs is due to a lower susceptibility of the species or to the less intensive contact between sheep and pigs in modern pig production systems cannot be determined. It may be speculated that the severe outbreak in the present herd may be attributed to a high virus load shed by the adolescent sheep (Li *et al.*, 2004) and/or to the intensive, direct contact between the sheep and the sows in the periparturient period.

There is some debate concerning the relation between the lambing period of sheep and MCF outbreaks in susceptible animals. For the wildebeest-associated MCF, it has been proven that virus shedding is at higher levels during periods of stress or parturition (Plowright *et al.*, 1960), but for the sheep-associated MCF, no correlation between parturition and shedding levels has been detected. Also, there is no significant amount of virus present in amniotic fluids or placental tissues (Li *et al.*, 2004). The rise in MCF incidence during lambing season as shown in epidemiological studies (Muller-Doblies *et al.*, 2001a) is probably unrelated to the parturition process itself but rather due to other factors such as the higher levels of virus shedding provo-

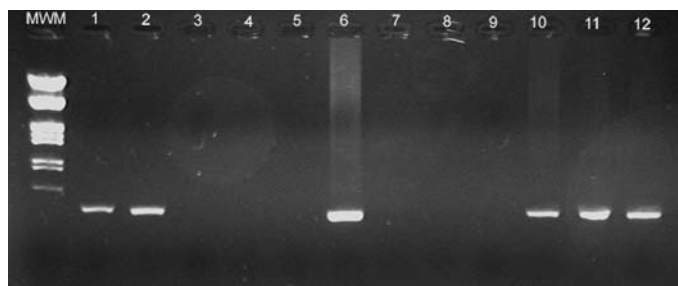


Figure 4. Agarose electrophoresis of the OHV-2 PCR products. MWM: molecular weight marker III (Roche). Amplification products from: lanes 1 and 2: affected sows; lane 3: negative control; lane 4: contact sow; lane 5: aborted fetus; lane 6: surviving sow; lane 7: contact sow; lane 8: aborted fetus; lane 9: contact sow; lanes 10 to 12: contact sheep.

ked by stress at high stocking densities, and the increased numbers of sheep coming into contact with MCF susceptible species.

Animals that are clinically affected with MCF are generally considered to be dead-end hosts (Mushi and Rurangirwa, 1981). Because of the high intensity contact between the sheep and the sows in the present herd and the fact that all the offspring of those sows could not come into direct contact with the sheep and were unaffected, there are no indications that porcine hosts of sheep-associated MCF are responsible for further horizontal spread. However, more research is needed to elucidate whether horizontal spread in pigs is possible.

The histopathological findings revealed that the animals had suffered from erosive lesions in the mucosae along the alimentary tract and from a generalized vasculitis compatible with MCF lesions. These findings were consistent with those previously described in pigs, including non-purulent meningo-encephalitis, gastritis, colitis and vasculitis in the skin (Loken *et al.*, 1998; Albini *et al.*, 2003).

In general, MCF is considered to be a disease with a mortality rate of between 95 and 100% (Smith, 1996). Up to now, no cases of recovered porcine MCF have been described. In the present study, 1 out of the 11 diseased sows recovered clinically, but then developed a persistent viremia with the sheep-associated MCF gammaherpesvirus and remained in poor body condition in the following months. The animal was culled for this reason, without the possibility for further sampling. The event of chronic or recovered cases of MCF has been described for cattle (O'Toole *et al.*, 1997), in which the animals become persistently infected and develop chronic arteriopathy.

Published records concerning the serological status of the sheep population for OHV-2 in Belgium are not available. Serological profiles in other European countries (Müller-Doblies *et al.*, 2001b, Fröhlich *et al.*, 2002) indicate that the virus is widespread in sheep flocks. The present case illustrates that, in terms of biosecurity, the mixing of sheep with susceptible animals implies a risk of developing MCF. Although pigs are raised mostly in intensive pig production systems, 12% of the pig producers in Belgium reported in a recent

survey on biosecurity that direct contact between sheep and pigs was occasionally possible in their herds (Ribbens *et al.*, 2008). This illustrates that incorporating MCF in the differential diagnosis of sudden death in pigs is not only theoretically important, but may also be of practical value for pig veterinarians.

CONCLUSION

This paper reports the first diagnosed case of OHV-2 infection in pigs in Belgium, and it shows that the virus can be transmitted from sheep to pigs. OHV-2 infection caused severe clinical symptoms and lesions, and led to a severe problem of sow mortality. Consequently, porcine MCF should be included in the differential diagnosis when facing problems of fever followed by death, especially when pigs are housed in close contact with sheep.

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