Neonatal foal death due to infection with equine arteritis virus in Belgium

Neonatale veulensterfte ten gevolge van een infectie met het equine arteritis virus in België

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ABSTRACT

This case report describes a small outbreak of neonatal foal death in Belgium due to infection with equine arteritis virus (EAV). The outbreak started with one foal suffering from acute dyspnea four days after birth. Despite intensive treatment, this foal died within a few hours. Three weeks later, another foal was born on the same farm in a healthy condition, although placental edema was found. At the age of 10 days, the foal showed acute respiratory distress and severe dyspnea and died shortly after. Post-mortem examination of both foals revealed consolidated diaphragmatic lung lobes with compensatory emphysema. Histologic lesions consisted of a mild acute interstitial pneumonia. Microscopic examination of the allantochorion from the second foal showed a focal necrotizing vasculitis, and immunolabeling demonstrated the presence of EAV antigens in chorionic vascular endothelial cells and macrophages. Upon inoculation of rabbit kidney (RK13) cell culture with a suspension of lung tissue, a cytopathic effect was observed. The agent was identified as equine arteritis virus by means of immunostainings.

SAMENVATTING

In deze casuïstiek wordt een beperkte uitbraak beschreven van neonatale sterfte in België, veroorzaakt door een infectie met het equine arteritis virus (EAV) bij twee veulens. De uitbraak begon met een veulen dat acute dyspneu vertoonde 4 dagen na de geboorte. Ondanks een intensieve behandeling stierf het veulen enkele uren later. Drie weken later werd op hetzelfde bedrijf een tweede veulen geboren dat gezond leek. Tijdens het onderzoek van de vruchtvliezen werd een verdikt oedemateus allantochorion vastgesteld. Op de leeftijd van 10 dagen werden acute ademhalings-symptomen en ernstige dyspneu waargenomen. Het veulen stierf voordat een behandeling kon worden ingesteld. Bij de autopsie werden bij beide veulens vast aanvoelende diafragmatische longkwabben en compensatoir emfyseem waargenomen. De histologische letsels waren indicatief voor een acute interstitiële pneumonie. Bij het microscopisch onderzoek van het allantochorion van het tweede veulen werd een focale necrotiserende vasculitis vastgesteld. EAV-antigenen werden met behulp van immunohistochemie aangetoond in endotheelcellen en macrofagen. Na de inoculatie van konijnenniercellen (RK13) met longsuspensie van beide veulens werd een cytopathisch effect waargenomen. Het agens werd met behulp van immunologische kleuringen geïdentificeerd als equine arteritis virus.

INTRODUCTION

Equine viral arteritis (EVA) is an infectious disease in horses caused by the equine arteritis virus (EAV), which is an RNA virus classified in the family of *Arteriviridae* (genus *Arterivirus*, order *Nidovirales*) (Cavanagh, 1997). Serological surveys have shown that EVA infections occur among horses in North and South America, Europe, Australia, Africa and Asia, with considerable variation in seroprevalence among countries and within equine populations (Timoney and McCollum, 1993).

The two most important modes of transmission are aerogenic and venereal. *In utero* transmission, transmission via secretions and transmission via indirect contamination have also been reported (Bryans *et al.,* 1975; Timoney and McCollum, 1993; Holyoak *et al.,* 2008). Within 2 days after infection, the virus spreads rapidly within the lungs and bronchial lymph nodes, followed by dissemination throughout the body via the

blood circulation (McCollum *et al.*, 1971; Henson and Crawford, 1974). A second cycle of infection and replication occurs in endothelial cells of internal organs, causing extensive endothelial damage (Bryans *et al.*, 1957).

Most infections are subclinical. Clinical signs during an outbreak vary widely between individual horses and between outbreaks (de Vries *et al.*, 1996). In adult horses, EVA infections frequently resemble other infectious respiratory diseases, especially those caused by equine herpes viruses 1 and 4 and equine influenza virus (Timoney and McCollum, 1993). Fever and leukopenia are the most consistently observed features, but others like abortion, systemic illness and persistent infection in accessory glands of the reproductive tract of stallions have also been reported (Bryans *et al.*, 1957; Holyoak *et al.*, 1993; Holyoak *et al.*, 2008). In neonates, sudden death, fever, depression, edema, hemorrhagic enteritis and/or severe respiratory distress have been described (Del Piero *et al.*, 1997; Del Piero, 2000).

In Belgium, a severe outbreak of equine viral arteritis abortion in an Arabian stud farm occurred in 2000 (van der Meulen *et al.*, 2001). Cases of neonatal foal death have not been reported in Belgium until now.

CASE HISTORY

The first foal was born after a normal gestation period. Complete placenta expulsion occurred after 3 hours. The foal received anti-tetanus serum (Intervet, Belgium) and prophylactic antibiotics (Cobactan® 4,5%, Intervet, Belgium). The immunoglobulin concentration in the serum of this foal was > 8g/l 16 hours after birth. Two days later, the mare showed an elevated temperature (39.8°C) and therefore the uterus was flushed with saline. The next day, the foal showed severe dyspnea, without fever. A treatment with ipratropiumbromide (Atrovent®, Boehringer Ingelheim NV, Belgium), ceftiofur (Excenel®, Pfizer, Belgium), oxygen and furosemide (Dimazon®, Intervet, Belgium) was started. No clinical improvement was seen and the foal died a few hours later.

At the same farm, a second healthy foal was born from an 8-year-old Belgian Warmblood mare after 346 days of pregnancy. Placenta expulsion occurred within 20 minutes. The placental weight was increased (8,950 kg) and edema was seen on the pregnant horn. No other placental abnormalities were noticed upon macroscopic examination. Samples from the pregnant horn, corpus and placenta at the level of the cervix were fixed in 10% formalin for histological examination. The foal received anti-tetanus serum (Intervet, Belgium) and prophylactic antibiotics (Cobactan® 4.5%, Intervet, Belgium) were given for 5 days. The concentration of immunoglobulins, which was checked 18 hours after birth, was higher than 8 g/l. Ten days later, the foal was found suffering from tachypnea and tachycardia and died before further clinical examination and veterinary treatment were initiated.

LABORATORY EXAMINATIONS

Gross examination

Post-mortem examination of both foals revealed enlarged, non-collapsed, congested and consolidated lungs with superficial rib imprints. Alveolar, interstitial and focal bullous emphysema was present in the craniodorsal lobes of the second foal. Samples from the lungs were fixed in 10% formalin for histology and immunohistochemistry. In addition, samples from the lungs and spleen were collected for bacteriological examination and samples from the lungs, spleen and liver were collected for virological examination.

Histological examination and immunohistochemistry

Fixed samples were embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (HE) according to standard techniques.

Histologically, a focal moderately necrotizing vasculitis was found in allantochorionic villi (Figure 1a). Adjacent to this lesion, a moderate infiltrate of neutrophils and lymphocytes was present. Multifocal co-
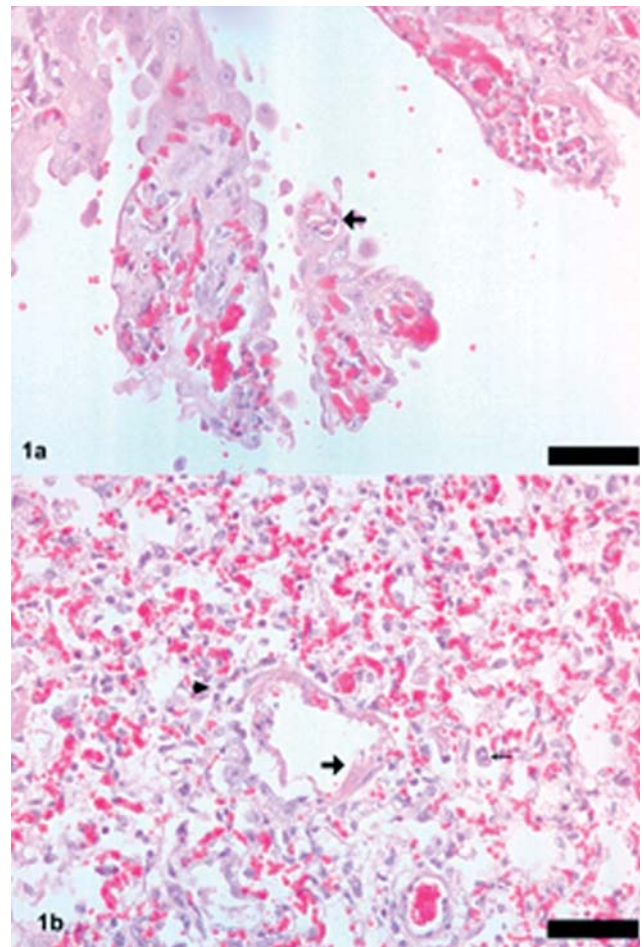

Figure 1. a: Necrotizing vasculitis of arteriole (arrow) in the chorion of the placenta. HE, bar 50 µm. b: Acute alveolar damage in lungs: hyaline membranes (large arrow) in bronchioli, hyperplastic type 2 pneumocytes (arrowhead) and activated pulmonary alveolar macrophages (small arrow). Bar 250 µm.
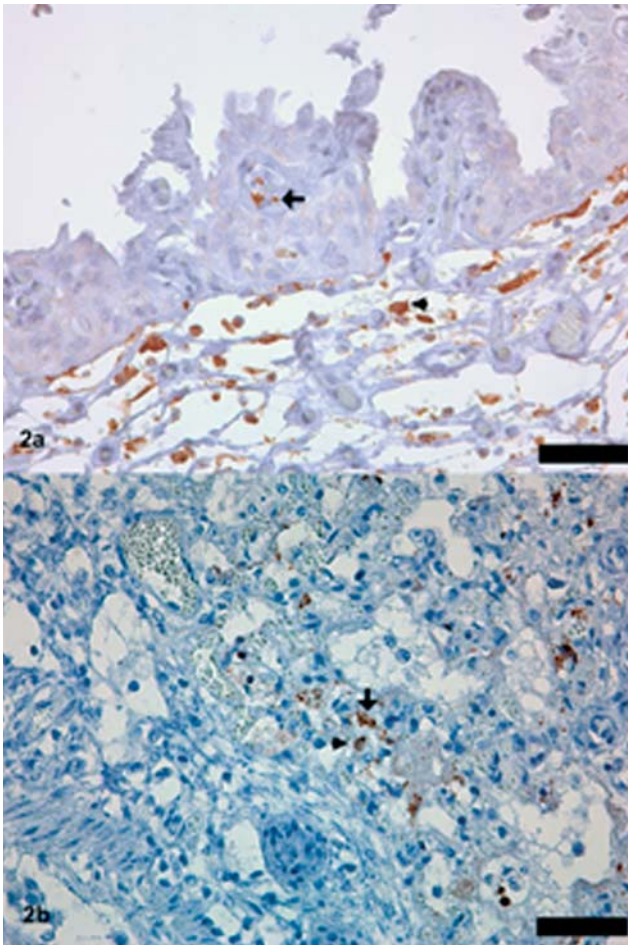
Figure 2. a: Positive immunolabeling for EVA in endothelial cells (arrow) and macrophages (arrowhead) in chorion of the placenta. Bar 50 µm. b: Positive immunolabeling for EVA in endothelial cells of alveolar capillaries (arrow) and pulmonary alveolar macrophages (arrowhead) in lungs. Bar 50 µm.

Figure 3. a: Cytopathic effect of EVA in RK13 cells at 7 days post inoculation. Rounding and vacuolization of the cells were observed. b: EVA-positive RK13 cells as revealed by immunofluorescence.

agulation necrosis of placental trophoblasts was observed upon microscopic examination. In the lungs of both foals, multifocal necrosis of alveolar and bronchiolar epithelial cells was present. Alveoles were often lined by hyaline membranes (Figure 1b). Overall, the number of alveolar macrophages was increased. Type II pneumocytes were hypertrophied and hyperplastic (Figure 1b). Occasionally, necrosis of the tunica media of medium-sized arteries was observed.

Paraffin sections from lungs and placenta were stained by immunohistochemistry using a mixture of three mouse monoclonal antibodies (mAbs): two mAbs were directed against the Gl protein and one mAb was directed against the M protein of EAV (van der Meulen et al., 2001). Staining was performed using Envision-mouse (Dako, Denmark). In addition, samples of liver and lungs were immunostained with biotinylated anti-EHV1 polyclonal antibodies (van der Meulen et al., 2003), followed by Envision+system-HRP (DAB) (Dako, Denmark) for exclusion of equine herpesvirus type 1 (EHV1) infection. Immunolabelling for EAV antigens was positive in the lungs of both foals and the placenta of the second
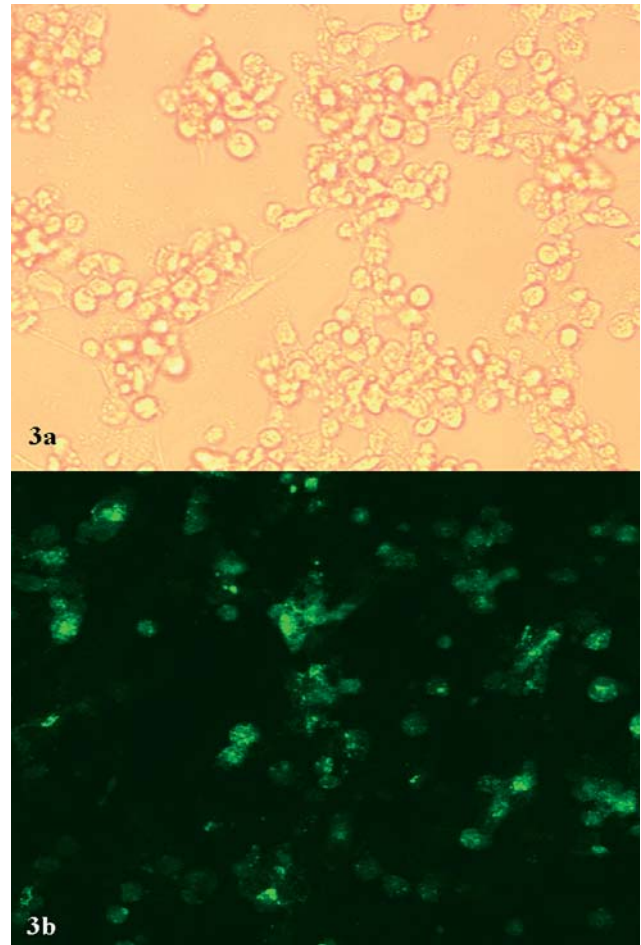
foal. Positive staining in allantochorion was restricted to patchy areas of vascular endothelium and macrophages in the lamina propria (Figure 2a). In the lungs, multifocal, ad random distributed areas of EAV positive cells were mainly localized in the endothelium of alveolar capillaries and occasionally in moderately-sized arteries, as well as in the alveolar macrophages (Figure 2b). EHV1 antigens were not demonstrated.

Bacteriological examination

Standard bacteriological and mycological examinations were performed on the lungs and spleen of both foals. Pathogenic bacteria were not isolated from any of the samples.

Virological examination

Suspensions (20%) of the lungs, spleen and liver were incubated for 1 hour with monolayers of RK 13 cells at 37°C in an atmosphere containing 5% CO_2. Afterwards, the inoculum was replaced by medium and

the cells were further incubated. Rounding and vacuolization of the RK13 cells were observed after 4 days of incubation, and after 7 days most of the cells were detached from their support (Figure 3a). No such changes were observed in the negative control cells.

An indirect immunofluorescence staining was performed on virus-infected RK13 cells and lung tissue using the three anti-EAV mouse mAbs, as described above. In a second step, tissues and cells were incubated with a goat anti-mouse antibody labeled with FITC (Molecular Probes, Eugene, OR, USA). EAV-positive antigens were found in the cytoplasm of virus-infected RK13 cells (Figure 3b) and in the cytoplasm of lung cells.

DISCUSSION

Although equine viral arteritis (EVA) has a worldwide distribution, few clinical cases have been reported in Belgium. In 2000, a severe outbreak of abortion occurred in a Belgian stud (van der Meulen *et al.,* 2001). The present study describes the first neonatal foal death caused by EAV in Belgium.

How the outbreak started remains elusive, as both foals appeared normal at birth. In the first case, the mare showed fever two days post-partum. The most common cause for fever after partus is an endometritis. However, in this case the placenta was completely expulsed and no macroscopic remains could be found during uteral lavage with saline, although the reliability of macropscopic evaluation can be questioned. No further examination of the placenta was performed. In the second case, the placenta was edematous and closer examination revealed a focal moderately necrotizing vasculitis in the allantochorionic villi. Such lesions suggest an infectious cause, and an *in utero* infection of the foal (Del Piero *et al.*, 1997). The hypothesis of an infectious etiology was confirmed by immunohistochemical staining, where EAV-positive macrophages and endothelial cells were demonstrated in the allantochorion. Transplacental infections with EAV during late gestation have been reported, but are not very common (Vaala *et al.*, 1992; Timoney and McCollum, 1993). Neonatal deaths associated with naturally acquired EVA have also been reported (Doll *et al.*, 1957; Golnick *et al.*, 1981), along with a sporadic, isolated case in a foal (Vaala *et al.*, 1992). Hence, to date, only a few reports of EVA outbreaks are available, primarily affecting neonates (Del Piero *et al.*, 1997; Del Piero, 2000). The histological findings in the lungs and allantochorion are in agreement with those reports.

Looking in retrospect, the outbreak described in this study probably started with an EAV infection in the first mare, which was introduced without quarantine to the farm to foal. The virus was most likely then transmitted by the respiratory route to the foal. The second mare probably got infected around the same period, either via the respiratory route or via indirect contamination, and it then passed the virus on, *in utero,* to its unborn foal. There were no records of other horses showing fever or other symptoms in the same stable at that time. Since the infections were mostly subclinical, other adult horses on the same farm might have got an EVA infection without being noticed. More than 40 foals were born healthy on the farm in the months following this outbreak.

The outbreak of EVA in neonates described in the present report serves as a warning about the necessity to include EVA in the differential diagnosis when foals are presented with fever, depression, edema, respiratory distress and/or sudden death. Since the prevalence of EVA in Belgium seems to be rising, more clinical outbreaks can be expected in the future. Although vaccines are available abroad, they are not yet registered for use in Belgium. The most important measures in the event of an outbreak in Belgium are management strategies such as quarantining new animals and eliminating EVA-positive breeding stallions from breeding populations (Holyoak *et al.*, 2008).

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