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Pulmonary artery haemorrhage in newborn calves following bluetongue virus serotype 8 experimental infections of pregnant heifers

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ABSTRACT

The emergence of bluetongue disease (BT) among livestock in Europe in 2006 raised many questions including the occurrence and epidemiological significance of foetal infections in cattle. To clarify these aspects, vaccinated and unvaccinated pregnant heifers were sequentially infected twice in an isolation facility (biosafety level 3) with a northern European outbreak strain of Bluetongue virus serotype 8 (BTV-8). The study was terminated 2 months after calving with necropsy of the dams and their offspring. The cattle were monitored throughout the study by clinical scoring and for the presence of circulating neutralising antibodies, and after calving for the presence of infectious virus and viral RNA in blood and milk. Four calves, one born from a vaccinated dam and three from non-vaccinated ones, that were infected at 120 days of gestation had obvious haemorrhage of the pulmonary artery at necropsy. Although haemorrhage of the pulmonary artery is highly characteristic of BT, viral RNA was not detected in any of these calves. Furthermore, although none of the calves born from heifers infected prior to mid-gestation had teratogenic BTV typical brain lesions, some had lesions at birth suggestive of *in utero* BTV infection. Despite the lack of viral RNA detection, the presence of haemorrhage of the pulmonary artery deserves to be reported as a new observation in the context of the multiple investigations having as main subject the BTV placental crossing in cattle.

1. Introduction

Bluetongue virus (BTV) causes the eponymous bluetongue disease (BT), belongs to the family *Reoviridae* and represents the type species of the *Orbivirus* genus (Mertens et al., 2005). BTV is an arbovirus transmitted to susceptible mammals, mostly wild and domestic ruminants, by the bite of haematophagous female midges of the *Culicoides* genus. The unexpected introduction of the so far exotic BTV serotype 8 (BTV-8) in the core of Western Europe in 2006 and its rapid spread, constituted the major sanitary event in animal health of the last years. In addition to a surprising severity of the clinical expression of the disease in cattle, abortions and nervous abnormalities were observed in the offspring of affected ruminants (Vercauteren et al., 2008). Foetal infection in cattle and sheep is long ago described (Schultz and Delay, 1955), but is very rarely related to wild type virus natural infection (Housawi

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et al., 2004). In the very vast majority of cases, laboratory adapted strains, like modified live vaccine viruses, are involved (MacLachlan et al., 2000). Placental crossing represents an additional feature increasing the considerable economic losses related to BTV-8 (Velthuis et al., 2010).

With regards to BT epidemiology in Europe, vaccination had been rapidly considered a strategic option inevitable to stop the spread of the disease, to control BT clinical outbreaks in endemic areas and to allow safe movement of animals (Zientara et al., 2010). Therefore, several Member States and Switzerland launched massive vaccination campaigns, starting in 2008 with unregistered monovalent inactivated vaccines under temporary use authorisation. Efficacy and safety qualities of the vaccines were assessed through large scale use (Eschbaumer et al., 2009; Gethmann et al., 2009). In March 2009, the European Medicine Agency granted in the European Union the use of one of these vaccines against BTV-8 (BTVPUR Alsap 8, Mérial, Lyon, France). In general, populations of Palaearctic *Culicoides* species raises in spring and peak in temperate regions in late summer (Takken et al., 2008). As a consequence, cattle are likely submitted to several natural infectious challenges during the same vector-active period.

A recent work (van der Sluijs et al., 2012) investigated the protection conferred by a commercial inactivated vaccine (Bovilis BTV-8, Intervet) against foetal infection in pregnant ewes and heifers. In that study, the animals were euthanized 3 weeks after BTV-8 inoculation, allowing the evaluation of the consequences of the infection after a short period from the virus exposition.

In our study, pregnant heifers, separated in three groups, vaccinated, non-vaccinated and control, were infected through two successive BTV-8 challenges. Inoculations were realised 4 months apart, in order to correspond to the times of the year with the highest vectorial activity. BTV-8 placental crossing and the efficacy of the vaccine were investigated. To further evaluate the modalities of trans-placental transmission, calves were monitored during 2 months after their birth regarding clinical, virological and serological parameters. The presence of BTV-8 induced malformations was particularly explored by an extensive necropsy of the offspring at the end of the experiment.

2. Materials and methods

2.1. Ethics statement

All the animals were confined for the whole length of the study in an insect-secure biosafety level 3 zone (BSL3) at the Experimental Infectiology Platform (PFIE) of the National Institute for Agronomic Research (INRA) – Research Centre of Tours (Nouzilly, France). Experiments were approved by INRA's Committee on the Ethics of Animal Experiments and conform to the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organisations of Medical Sciences.

2.2. Animals and group constitution

Twelve Holstein heifers aged between 1 and 2 years were used. All the animals were tested seronegative and non viraemic for BTV and bovine viral diarrhoea virus (BVDV), and seronegative for bovine herpes virus 1 (BoHV-1), *Neospora caninum* and *Coxiella burnetii*. A thorough general clinical examination was carried out on all the animals by a veterinarian before including them to the study, to confirm their asymptomatic state, in accordance to physiological standards (Jackson and Cockcroft, 2002).

Heifers were randomly separated in three fence-isolated groups. These 3 groups were constituted as following: 5 animals in the non-vaccinated group (identified further as NV); 5 animals in the vaccinated group (V) and 2 animals in the control group (C). When an individual animal is mentioned, it is referred as its group name followed by its identification number. Once the heifers gave birth, their offspring was named as "O" followed by the vaccination status of the mother (NV, V or C) and a number depending on the chronologic order of calving within its group. When necessary the identification number of the dam was added in brackets.

The vaccinated heifers received for both injections of the vaccination protocol a 1 ml BTVPUR AlSap 8 (Merial, Lyon, France) dose, subcutaneously in front of the left shoulder. Heifers were vaccinated following the manufacturer's recommendations, with a first injection 78 days before the first infection (−78 dpi) and revaccinated 28 days later (−50 dpi). The first infectious challenge took place 50 days after second vaccine injection (referred later as 0 dpi) and the second challenge at 121 dpi (Fig. 1).

All animals were introduced in the BSL3 facility 1 week before the beginning of the experiment to allow their acclimatisation. Euthanasia was realised by intravenous injection of pentobarbital sodium (Dolétal, Vétoquinol, Lure, France), 10 g/animal, followed by bleeding once the corneal reflex disappeared. Heifers and their offspring were euthanatized at 223 dpi and necropsied (Fig. 1).

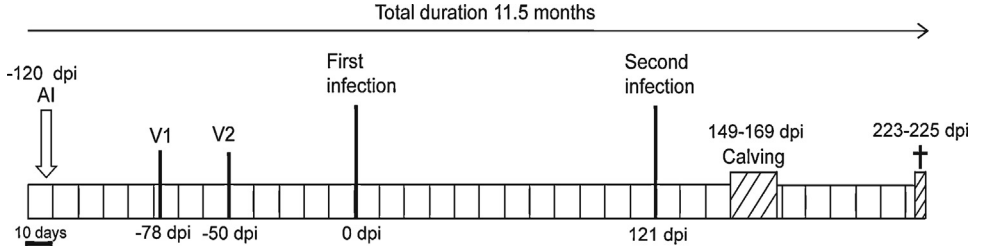

Fig. 1. Experimental timeline. Successive vaccinations (V1 and V2) and infections are represented. Artificial insemination (AI), calving period and euthanasia (†) are also represented.