Evidence for transplacental transmission of the current wild-type strain of bluetongue virus serotype 8 in cattle

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DURING the winter 2007/08, an outbreak of unprecedented development lesions of the central nervous system was detected in newborn or stillborn calves and lambs among the routine submissions to the Faculty of Veterinary Medicine, Liège, Belgium, for postmortem examination. The congenital malformations ranged from brains with thin-walled cerebral hemispheres, variably sized cerebral cysts and dilated lateral ventricles (porencephaly with some degree of hydrocephalus) to brains in which the cerebral hemispheres were represented only by fluid-filled sacs (hydranencephaly). The brainstem structures were usually present but the cerebellum was sometimes dysplastic, cystic or replaced by a fluid-filled sac (Fig 1).

As the routine cases for postmortem examination in 2007 had been collected in exactly the same way as cases since 1989, the outbreak could not be explained by a recruitment bias. Rather, the incident was considered to be associated with the emergence of bluetongue virus (BTV) serotype 8 (BTV-8) in northern Europe in 2006 (Thiry and others 2006) and its subsequent dissemination across Europe in 2007 (Enserink 2008). Experimental and spontaneous cases of BTV-induced brain malformations have been reported in both cattle and sheep. For example, direct injection of the virus into the bovine fetus in utero resulted in the birth of calves with severe brain dysfunction due to porencephaly or hydranencephaly (Barnard and Pienaar 1976, MacLachlan and others 1985, Thomas and others 1986); experimental infection of pregnant dams with several serotypes of BTV has been reported to cause similar defects in their offspring (Osburn 1972, Parsonson 1990, Flanagan and Johnson 1995). In view of these reports in the literature, and in the context of the dissemination of BTV-8 across Europe in 2007, the observation of this series of congenital brain malformations at postmortem examination could have been expected.

Evidence of transplacental transmission of BTV causing brain malformations in the offspring has, so far, been recorded after the infection of pregnant dams with laboratory-adapted, in particular those that are vaccine attenuated. Moreover, spontaneous occurrences have been described only in the USA and South Africa, countries in which live attenuated strains were released (Luedke 1985, Parsonson 1990, MacLachlan and others 2000). In contrast, malformations in the offspring were not reported when pregnant dams were infected with wild-type strains (Flanagan and others 1982, Groocock and others 1983, Parsonson and others 1994), or in pathological descriptions of bluetongue disease in ruminants associated with spontaneous infections outside the USA or South Africa (Davies 1980, Herniman and others 1980, Prasad and others 1992). Therefore, if the current European BTV-8 strain indeed infects fetuses, as suggested by the cerebral malformations observed by the authors, its biology would differ significantly from that of wild type BTV strains studied previously.

To confirm, and examine the importance of, transplacental transmission of the current BTV-8 strain under field conditions, a total of 110 pairs of serum samples from cows and their calves were collected during caesarean sections,

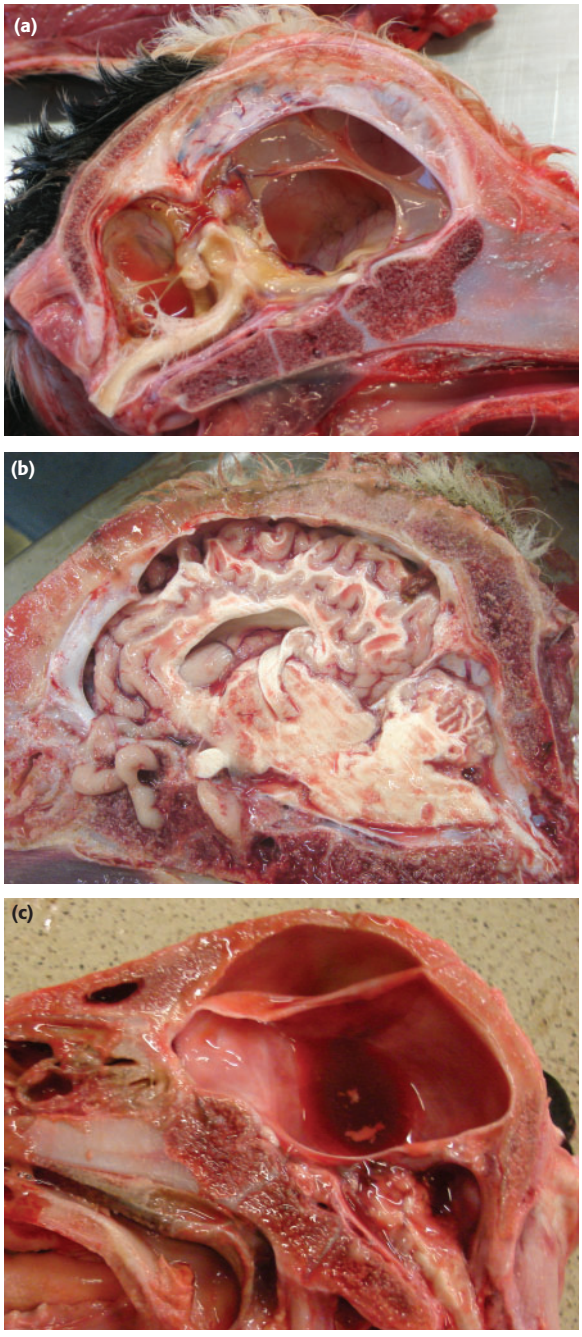

FIG 1: (a) Hydranencephaly in a 48-hour-old calf examined postmortem on February 22, 2008. The cerebral hemispheres are replaced by a thin-walled, fluid-filled cyst. The brainstem is preserved but not the cerebellum, which appears as a tiny bud. (b) Porencephaly in a two-week-old calf examined postmortem on February 23, 2008. Variably sized cerebral cysts in the grey matter are clearly visible, and the lateral ventricle is dilated. (c) Hydranencephaly in a stillborn calf examined postmortem on January 23, 2008. Remnants of the cerebral hemispheres appear as a 4 mm thick wall enclosing a single fluid-filled cyst. The brainstem is preserved but not the cerebellum

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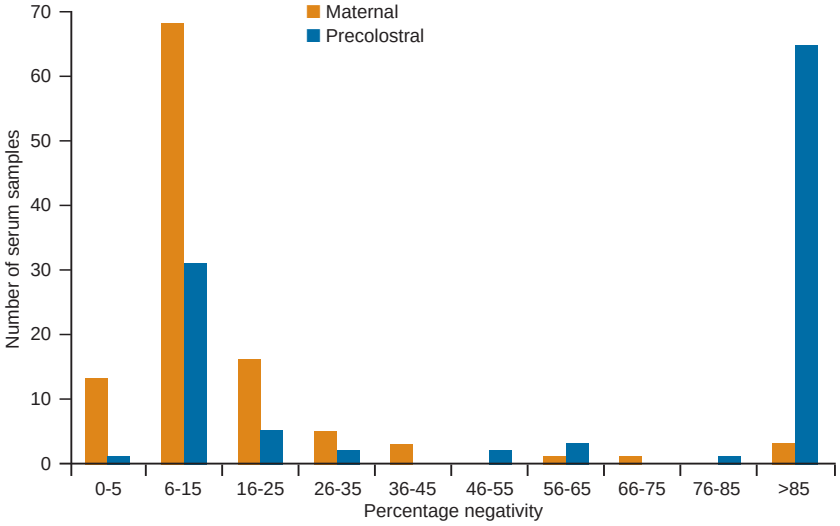

FIG 2: Frequency distribution of the results of a competitive ELISA for antibodies to bluetongue virus on 110 paired maternal and precolostral calf sera sampled between November 22, 2007, and January 31, 2008, in 65 randomly selected farms in southern Belgium. Sera with a percentage negativity value, relative to the negative control in the assay, of below 35 were considered positive

between November 22, 2007 and January 31, 2008. The maternal and precolostral calf serum samples were taken from clinically healthy animals on 65 randomly selected farms in southern Belgium. The sera were examined for anti-BTV antibodies using the ID Screen Bluetongue Competition assay (ID.VET) according to the manufacturer's instructions. This assay is based on the detection of antibodies specific to the highly conserved VP7 protein of BTV and is therefore designed to detect infection by any subtype of BTV. The results were expressed as percentage negativity (PN) compared with the negative control in the kit, and translated to a positive, doubtful or negative result according to the cut-off values provided by the manufacturer (PN<35 positive, 35<PN<45 doubtful, PN>45 negative). The assay has been reported to have a diagnostic specificity of 98·2 per cent (95 per cent confidence interval [CI] 97·1 to 100 per cent) and a sensitivity of 87·8 per cent (95 per cent CI 85·1 to 91·1 per cent) (Vandenbussche and others 2008). The results of the ELISA screening are shown in Table 1. To illustrate the antibody reactivity patterns, Fig 2 shows a frequency distribution analysis, given as a profile distribution of the PN values divided into 10 classes. The distribution profile of the results obtained from the 110 maternal sera showed a quasi-unimodal, positively skewed shape (93 per cent of the cows were seropositive), whereas the results from the 110 precolostral calf sera generated a clear bimodal profile, with distinct negative and positive categories (35 per cent of the calves were seropositive). Similar to the profile obtained by Vandenbussche and others (2008) in

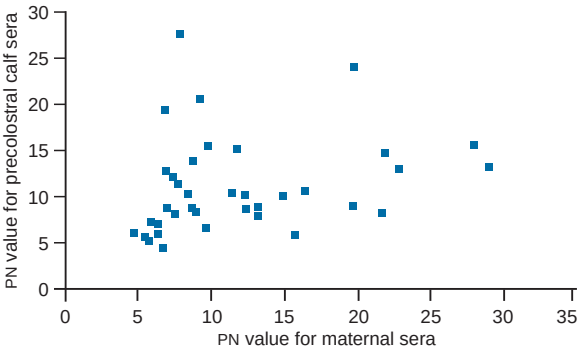

FIG 3: Comparison of the competitive ELISA results among the 38 double-positive paired maternal and precolostral sera. The correlation was not significant. PN Percentage negativity

TABLE 1: Results obtained by a competitive ELISA for antibodies to bluetongue virus in paired serum samples from 110 calves before they received colostrum, and from their dams

	Precolostral calf sera			
Maternal sera	Positive	Doubtful	Negative	Total
Positive	38	0	64	102
Doubtful	1	0	2	3
Negative	0	0	5	5
Total	39	0	71	110

January 2007, at the end of the first European epidemic of BTV-8, which started in 2006, only a very small number (n=3) of maternal sera had PN values that fell between the positive and negative classes, indicative of the low or absent virus transmission by the vector midges at that time of year. As the intact, epitheliochorial type placenta of ruminants theoretically does not allow maternal to fetal transfer of immunoglobulins (Latshaw 1987), and because even BTV-infected bovine placentomes are not reported to show histological modifications suggestive of trophoblastic, epithelial or vascular necrosis (Hubbert and others 1972), passive transfer of maternal antibodies is unlikely. This conclusion is reinforced by the fact that the correlation between the PN values of the paired maternal and precolostral calf sera was not significant (Fig 3), and also by the detection of 64 seronegative calves born to strongly positive dams. The results therefore unambiguously show that 35 per cent of the calves sampled were infected by BTV-8 in utero. To the authors' knowledge, there are no previous reports of calves born seropositive to dams infected with wild-type strains of BTV; in contrast, experimental infections of pregnant dams with various wild-type BTV strains systematically resulted in seronegative offspring (Flanagan and others 1982, Richardson and others 1985, Parsonson and others 1987), and seropositive calves have been detected only after pregnant dams were infected with cell-adapted (vaccine attenuated) strains (Gibbs and others 1979, Richardson and others 1985, Flanagan and Johnson 1995).

In conclusion, the observation of a series of hydranencephalopathies in calves and lambs examined postmortem, and the anti-BTV-8 antibody titres measured in paired sera from a cohort of precolostral calves and their dams, strongly suggest that the BTV-8 strain that spread across Europe in 2007 differs from previous wild-type strain in that it is able to cross the ruminant placenta spontaneously and infect the fetus. As this unexpected transplacental transmission occurred quite frequently, in approximately 37 per cent of pregnancies in the animals studied, the current epidemic could have a more important economic impact than would be anticipated from the literature available, due to more frequent abortions and the birth of non-viable offspring.

The epidemiological modelling of the spread and overwintering of the current BTV-8 strain must take this atypical biology into account because the virus might persist in offspring after birth, thus providing biting vector midges with a fresh source of virus during the spring. It is also of crucial importance to determine whether the vaccines developed in the future effectively prevent transplacental transmission, and to examine the possibility that a proportion of the offspring could be immunotolerant and remain persistently infected.

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