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Short communication

Colostrum transmission of BTV-8 antibodies from dairy cows six years after vaccination

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ABSTRACT

Bluetongue virus (BTV) antibodies were analysed in 27 Swiss calves born in 2016 at the age of 16–19 days using competitive enzyme-linked-immunosorbent-assay (cELISA) and virus neutralization test (VNT) (animal trial permission number: 75684). Obligatory documentation proved that 15 of 27 dams were BTV-8 vaccinated once or three times in 2008–2010. The offsprings of the non-vaccinated dams were seronegative. Two of three calves and 11 of 12 calves descending from dams who had been vaccinated one or three times, respectively, had BTV specific serum antibodies. As Switzerland is considered BTV-free from 2010 to 2016, it is likely that BTV-8 antibodies were transferred via colostrum. Furthermore, we confirmed neutralizing cross-reactivity of BTV-8 with BTV-4 antibodies as 5 samples positive for BTV-8 were also reactive with BTV-4 antibodies.

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1. Introduction

The occurrence of infections with Bluetongue virus (BTV) (family *Reoviridae*, subfamily *Sedoreovirinae*, genus *Orbivirus*) in 2006 in Northern Europe led to severe losses [1–3]. Due to the first cases of Bluetongue disease (BTD) in 2007 in Swiss cattle [4], compulsory vaccinations were established in Switzerland from 2008 to 2010 [3,5]. In 2008, cattle had to be vaccinated earliest starting at an age of 3 months with prescribed BTV serotype 8 (BTV-8) vaccines. In 2009 and 2010, compulsory vaccinations had to be done until end of May followed by booster vaccinations within 3–8 weeks after the first injection [6]. Since the last confirmed BTD case in

2010, Switzerland was considered free from BTV [7], but every summer, the risk for new introductions via insects (*Culicoides* spp.) or imported cattle [8,9] is evident. In autumn 2017 only two cases of BTV-8 infections in Swiss cattle were reported [10]. Currently, vaccination is voluntary in Switzerland.

It is known that BTV antibodies can persist in cattle at least four years after vaccination [11–14]. In the frame of a clinical study in calves, BTV specific antibody titres were measured using competitive enzyme-linked-immunosorbent-assay (cELISA) and virus neutralization test (VNT).

The unpredicted results regarding the presence of BTV serum antibodies in calves in 2016 descending from dairy cows who had been vaccinated with BTV-8 the last time in 2010 will be reported in this short communication.

2. Material and methods

We report our findings according to the ARRIVE guidelines [15]. The trial was performed on a Swiss organic dairy farm in the Aargau Canton at 480 m above sea level between June and November 2016. The clinical trial, within our findings were gained, was

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approved by the Aargau cantonal veterinary office (animal experiment permission number: 75684) and was conducted according to the Swiss law on animal welfare and the EU directive 2010/63/EU for animal experiments. A group of 27 calves (inclusion criteria: no calves from heifers, birth weight 35–53 kg, adequate colostrum intake (4 L during the first 8 h), and 52 g/L total protein in the serum (measured in 2–4 days old calves)) was housed individually in outdoor calf huts with straw bedding.

Blood samples for serology were taken between the 16th and 19th day post-partum from disinfected jugular vein with single-use needles (18G, 1.20 × 40 mm). The samples were kept at room temperature for 30 min to assure adequate coagulation before they were stored and transported in a 8 °C cooling box to the laboratory of the Research Institute of Organic Agriculture in Frick (Switzerland) for centrifugation (10 min at 2000g at 24 °C), separation of sera and freezing (–20 °C).

To investigate the BTV antibody status of dams who were still alive in October 2017 (n = 14), blood samples were taken from the tail vein. Sample processing and testing for BTV antibodies were conducted similarly to those obtained from the calves.

2.1. Serology

2.1.1. Enzyme-linked-immunosorbent-assay (ELISA)

The sera were tested for the presence of antibodies against BTV using the commercially available cELISA (VMRD, Pullman, WA, USA) according to the manufacturer's protocol. Samples were considered positive and negative when the percentage of inhibition was less than 50% and higher than 60%, respectively; samples $\geq 50\%$ and $\leq 60\%$ were assessed as inconclusive.

2.1.2. Virus neutralization test (VNT)

To detect neutralizing antibodies against BTV, VNTs using either BTV-4 or BTV-8 were performed according the method described

in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (<http://www.oie.int/international-standard-setting/terrestrial-manual/>). Briefly, sera were inactivated for 30 min at 56 °C using a dry block with ceramic beads. Thereafter, 75 μ l medium were added to the first row of a 96 well flat-bottomed microtiter plate, 50 μ l were pipetted in the remaining wells. Subsequently, 25 μ l of serum were added to the first row and two-fold dilutions were performed. A virus concentration of 100 TCID₅₀/50 μ l was added to the mixture of serum and medium, and the plates were incubated for 1 h at 37 °C and 5% CO₂ in an incubator. 50 μ l of BHK-21 cell suspension was then added to the incubated samples and were incubated using the same conditions. Subsequently, the cells were examined daily on the three consecutive days for the presence of cytopathic effects.

2.1.3. Virus strains

BTV-4 (BUL2014/16) and BTV-8 (17/12/82) virus strains were kindly provided by the Friedrich-Loeffler-Institut, FLI, Riems, Germany and the Pirbright Institute, UK respectively.

3. Results

Thirteen of the 27 calves showed BTV antibodies in cELISAs and VNTs 16–19 days post-partum (Table 1) in the serum samples taken during summer 2016. Investigations of obligatory documentation of BTV vaccinated cattle proved that 15 of the 27 dams were vaccinated once (n = 3) or three (n = 12) times in the years 2008–2010 according to the Swiss law. No calves of non-vaccinated dams (n = 12) were positive for BTV antibodies. None of the nine non-vaccinated dams still available for serology in 2017 was positive for BTV antibodies.

11 calves of the 12 triple vaccinated dams were positive for BTV antibodies in the cELISA and, if cytotoxicity did not hinder the interpretation, also in the VNT (Table 1). The two dams that had

Table 1
Birth dates of calves, vaccination status and BTV antibody titres of calves and dams.

ID calf	Date of birth calf	ID dam	Date of birth dam	Compulsory vaccination (BTV-8) dam			Dam October 2017		Calf 16–19 days old (June–October 2016)		
				2008	2009	2010	VNT BTV-8	VNT BTV-4	cELISA	VNT BTV-8	VNT BTV-4
5498	06.08.2016	12	18.09.2003	yes	yes	yes	–	–	1:16	1:20	ni
5492	21.07.2016	112	28.12.2004	yes	yes	yes	–	–	neg	neg	neg
5477	12.06.2016	145	05.07.2005	yes	yes	yes	–	–	1:16	1:20	1:14
5484	24.06.2016	111	26.09.2005	yes	yes	yes	1:20	neg	1:128	1:22	ni
5480	17.06.2016	73	17.03.2006	yes	yes	yes	–	–	1:32	1:20	1:10
5503	05.09.2016	8	10.12.2007	yes	yes	yes	–	–	1:32	1:22	ni
5491	17.07.2016	15	14.04.2008	yes	yes	yes	–	–	1:16	ni	ni
5486	26.06.2016	135	08.09.2008	yes	yes	yes	–	–	1:4	1:22	1:6
5495	31.07.2016	123	05.10.2008	yes	yes	yes	–	–	1:8	1:16	ni
5483	23.06.2016	147	06.10.2008	yes	yes	yes	1:12	neg	1:16	1:20	1:6
5501	28.08.2016	94	22.10.2008	yes	yes	yes	–	–	1:16	ni	ni
5502	28.08.2016	84	28.11.2008	yes	yes	yes	–	–	1:128	ni	ni
5506	13.09.2016	121	17.09.2009	na	na	yes	1:6	neg	neg	1:8	neg
5487	26.06.2016	143	21.02.2010	na	na	yes	neg	neg	1:8	1:22	1:12
5489	09.07.2016	25	27.02.2010	na	na	yes	neg	neg	neg	neg	neg
5488	27.06.2016	149	06.06.2010	na	na	na	–	–	neg	neg	neg
5497	05.08.2016	70	29.11.2010	na	na	na	neg	neg	neg	neg	neg
5500	11.08.2016	107	09.02.2011	na	na	na	neg	neg	neg	neg	neg
5496	01.08.2016	132	28.06.2011	na	na	na	neg	neg	neg	neg	neg
5476	06.06.2016	110	30.08.2011	na	na	na	neg	neg	neg	neg	neg
5478	12.06.2016	62	25.10.2011	na	na	na	neg	neg	neg	neg	neg
5482	22.06.2016	27	23.04.2013	na	na	na	–	–	neg	neg	neg
5481	22.06.2016	27	23.04.2013	na	na	na	–	–	neg	neg	neg
5494	30.07.2016	4	14.05.2013	na	na	na	neg	neg	neg	neg	neg
5507	08.09.2016	40	30.05.2013	na	na	na	neg	neg	neg	neg	neg
5490	09.07.2016	3	29.06.2013	na	na	na	neg	neg	neg	neg	neg
5499	09.08.2016	117	29.06.2013	na	na	na	neg	neg	neg	neg	neg

The titres given are the highest dilution that was still positive in the ELISA; na: not analysed; dam was not yet born in the respective year; neg: negative; ni: not interpretable because of cytotoxicity; –: dam was not alive anymore.

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