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Short communication

BTV antibody longevity in cattle five to eight years post BTV-8 vaccination

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ABSTRACT

The Bluetongue virus serotype –8 (BTV-8) epizootic in Germany (2006–2008) was successfully eradicated, essentially by the massive application of commercially available inactivated BTV-8 vaccines. While a six-year antibody longevity of BTV antibodies post BTV-8 vaccination in cattle has been described previously, our study investigated the BTV-8-vaccine antibodies in cattle for up to eight years. In total, 157 bovine serum samples were analysed for the presence of group-specific BTV antibodies in both a commercial cELISA, and a BTV-8- specific serum neutralization test. A robust number of cattle were seropositive for group- and serotype-specific neutralising antibodies for five or more years. In selected animals, born and vaccinated in 2009 or later, the presence of BTV antibodies for up to eight years post BTV-8 vaccination could be confirmed. Our data also show, that booster vaccination prolonged the antibody longevity of vaccine-induced antibodies and the number of serologically positive cattle.

1. Introduction

In 2006, BTV-8 occurred in northern Europe for the first time [1]. The BTV-8 outbreak in Germany between 2006 and 2008 was controlled by an obligatory vaccination program, and caused enormous economic damages [2]. The last BTV-8 case was reported in Germany in November 2009 [3]. Based on the successful eradication program, the vaccination strategy changed from obligatory to voluntary in January 2010 [2,4]. On February the 15th, 2012, Germany declared itself free from BTV [2].

Over the last years, several studies investigating the antibody longevity of BTV-specific vaccine antibodies were published. In a study performed in Bavaria, 110 cattle sampled 4 weeks post initial vaccination (basic immunisation) in 2008 were tested with a cELISA with a seroprevelance rate of 82%, whereas only one out of ten cattle pre-selected for the serum neutralization test (SNT) showed serotype-specific neutralizing antibodies. After revaccination in 2009, 28 out of 28 pre-selected cattle were also positive in the BTV-8-specific SNT with a median titer of 20 [5]. Furthermore, a British study with 40 cattle with a basic immunization status revealed that group-specific antibodies were present in 95%, and serotype-specific neutralizing antibodies in 97.5% of the cattle for at least three years post vaccination [6]. Subsequently, the follow up study with 29 animals of the cattle group demonstrated a 97% seropositivity rate of both group and neutralizing BTV-8 antibodies four years post vaccination [7]. A further study showed a six-year antibody longevity of BTV-8 group- and serotype-specific neutralising antibodies post natural BTV-8 infection in twelve cows [8] and another study demonstrated BTV-8 neutralizing antibodies even six years post vaccination in dams, and the transmission of colostral neutralizing BTV-8 antibodies to their calves [9]. In addition, a seroprevalence study in France in 2016 suggested the antibody longevity of BTV group-specific antibodies for at least 5 to 6 years after natural infection or vaccination [10].

Our study investigated the long-term humoral immune response following BTV-8 vaccination. Since "fresh" BTV infections can be excluded in Germany since 2010 until the end of 2018, we were able to investigate the presence of group and neutralising BTV-8 antibodies in cattle for up to 8 years. Furthermore, the immune response after initial basic immunization and several booster vaccinations were tested. The presented data about antibody longevity of BTV-8 vaccinated cattle provides important information especially for diagnostics and epidemiological analyses.

2. Material and methods

2.1. Animals

The study included serum samples from 157 cattle of different breeds, born and vaccinated in Germany between 2009 and

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2012. The registration of all BTV vaccinations in Germany in the national database for identification and registration of animals (HI-Tier) is mandatory [3]. Only cattle with a completed basic immunization (initial two shots) and cattle with up to three annually booster vaccinations were integrated in the study. All samples were taken 5 to 8 years after the last BTV-8 vaccination during routine controls. Hence, sampling time points were documented in quarterly periods, but not precisely per month. The used vaccines were BLUEVAC®8 (CZ Veterinaria S.A., Spanien), Zulvac®8 (Fort Dodge, The Netherlands), BTVPUR® AlSap8 (Merial, Frankreich), and Bovilis BTV8 (Intervet Deutschland GmbH). We presume that the local veterinarians performed all vaccinations according to the manufacturers' instructions. In detail, the animals were categorized into four groups (5, 6, 7 and 8) according to the time span in years between the last BTV-8 vaccination and the sampling. Cattle with 4 years and 5 months up to 5 years and 4 months between the last BTV-8 vaccination and sampling were combined in group 5. The assignment to the groups 6, 7 and 8 were handled in a similar way using the rounded time intervals between BTV-8 vaccination and sampling, respectively. Ninety-two of the 157 cattle received the initial BTV-8 basic immunisation (vaccination status = 1). Additionally, within one year after the basic immunisation 53 cattle received one boost (basic immunisation plus one boost; vaccination status = 2). Within the following second year past basic vaccination, 10 cattle received a second boost vaccination (basic immunisation plus two boosts; vaccination status = 3) and 2 cattle received the basic immunisation plus three boosts (vaccination status = 4).

2.2. Serology

The 157 serum samples were screened for group specific antibodies using a commercial cELISA (ID Screen® Bluetongue Competition, ID-Vet, France) according to the manufacturer's instructions.

A serum neutralization test was performed for detection of serotype 8-specific neutralizing antibodies according to the standard protocol of the EU Reference Laboratory for BT (The Pirbright Institute, UK) [11]. The used virus stock was based on a German BTV-8 isolate of 2008 with a titre of $10^{5.83}$ $TCID_{50}$/ml. A positive serotype-specific BTV-8 serum and a negative reference serum were used as controls. Briefly, the sera were diluted in log2-steps (1:10–1:1280), and titrated against 100 $TCID_{50}$ of the BTV-8 virus. Plates were incubated for 1.5 h at 37 °C before overnight incubation at 4 °C. The following day, 100 μl of a Vero cell suspension with 30 000 cells/ml were added per well. After incubation for 5–6 days at 37 °C, all wells were stained with crystal violet and scored for a cytopathic effect (CpE). The neutralization titer was determined as the dilution of serum giving a 50% neutralization and was calculated according to the method of Spearman and Kärber [20]. Samples with a neutralizing antibody titre of $\geq \log_{10}$ 1 were considered as positive.

3. Results

The ELISA and SNT results of all 157 cattle were analysed according to two variables: (i) the approximated time spans of 5–8 years (groups 5–8) between the latest BTV-8 vaccination and the time point of sampling, and (ii) according to the respective number of boost vaccinations (basic immunisation plus one, two or three booster vaccinations). The serological results are summarised in Table 1 and Fig. 1, statistics in supplementary Table S1. In total, 111 samples (70.7%) were positive in the cELISA, and 128 samples (81.5%) in the SNT. Group-specific antibodies were found in group 5 in 23 cattle (71.9%), in group 6 in 45 cattle (66.2%), in group 7 in 35 cattle (81.4%) and in group 8 in 8 cattle (57.1%). With the BTV-8 SNT, neutralizing titres ranged from $\log_{10}$ 1.15 – to 2.81. BTV-8 seropositive cattle were detected in group 5 in 30 cattle (93.8%), in group 6 in 54 cattle (79.4%), in group 7 in 36 cattle (83.7%) and in group 8 in 8 cattle (57.1%). Sorted by the number of vaccinations, antibodies were detected with the ELISA in 55 animals (59.8%) having received the basic immunization only, in 46 animals (86.8%) with one boost vaccination, in 8 animals (80%) with two boost vaccinations and in two

Table 1
Overview of all tested bovine serum samples in the cELISA and the BTV-8-SNT, sorted according to their group (group 5–8 represents the approximated time span of 5–8 years between the last BTV-8 vaccination and time of sampling), and the number of received vaccinations.

Group°	Number of cattle	Positive in ELISA (%)	Positive in SNT (%)	Number of V.*	Number of cattle	Positive in ELISA (%)	Positive in SNT (%)
5	32	23 (71.88%)	30 (93.75%)	1	13	7 (53.85%)	11 (84.62%)
				2	19	16 (84.21%)	19 (100%)
6	68	45 (66.18%)	54 (79.41%)	1	43	24 (55.81%)	32 (74.42%)
				2	16	14 (87.5%)	14 (87.5%)
				3	7	5 (71.43%)	6 (85.71%)
				4	2	2 (100%)	2 (100%)
7	43	35 (81.40%)	36 (83.72%)	1	24	18 (75%)	18 (75%)
				2	16	14 (87.5%)	15 (93.75%)
				3	3	3 (100%)	3 (100%)
8	14	8 (57.14%)	8 (57.14%)	1	12	6 (50%)	6 (50%)
				2	2	2 (100%)	2 (100%)
5–8	157	111 (70.70%)	128 (81.53%)	1	92	55 (59.78%)	67 (72.83%)
				2	53	46 (86.79%)	50 (94.34%)
				3	10	8 (80%)	9 (90%)
				4	2	2 (100%)	2 (100%)

° Groups 5–8: Approximated time (5–8 years) between the last received BTV-8 vaccination and sampling time. Group 5 (approx. 5 years) = 4 years 5 months to 5 years 4 months, in group 6 (approx. 6 years) = 5 years 5 months to 6 years 4 months, in group 7 (approx. 7 years) = 6 years 5 months to 7 years 4 months, in group 8 (approx. 8 years) = 7 years 5 months to 8 years 4 months.

* Number of vaccinations: 1 = basic immunised cattle; 2 = basic immunised cattle + one boost (boost vaccination one year past basic immunisation); 3 = basic immunization + two boosts (boost vaccination one year past basic immunisation + one boost within the second year past basic immunisation); 4 = basic immunization + three boosts (boost vaccination one year past basic immunisation + two boosts within the second year past basic immunisation).

animals (100%) with three boost vaccinations. Positive SNT results were found in 67 cattle (72.8%) with only the basic immunization, in 50 cattle (94.3%) with one boost, in 9 cattle (90%) with two boosts and in 2 cattle (100%) with three boosts. The Fisher's exact test was applied to examine any statistically significant difference between basic immunised (V = 1) and revaccinated cattle (V = 2–4) and between positive and negative serological result in SNT. The test revealed that the revaccination significantly ($P < 0.05$) improved the serological results in the SNT.

Of the BTV antibody-positive cattle, 17 animals had mismatches between the cELISA and the SNT results. In all 17 mismatch cases, the samples were negative in the cELISA but positive in the BTV-8-specific SNT with titres between $\log_{10}$ 1.15–2.05 (Fig. 2). 29 of all the 157 cattle were seronegative in both the cELISA and the SNT (Table 2). These samples were from 2 cattle (6.3%) of group 5, 14 cattle (20.6%) of group 6, 7 cattle (16.3%) of group 7 and 6 cattle (42.9%) of group 8. Among these seronegative cattle were 25 cattle (27.2%) having received basic immunization, three cattle (5.7%) with one boost, one cattle (10%) with two boosts and no cattle with three boosts.

Twenty-one of all 157 cattle showed negative ELISA results with S/N values higher than 90. Fifteen of these cattle were also negative in the BTV-8-specific SNT, among them 14 cattle with the basic immunization only, and 1 cattle with a single booster vaccination. The six cattle with ELISA S/N values higher than 90, but positive BTV-8-SNT results had low titres ranging between $\log_{10}$ 1.15–1.60 and all had received the basic immunization only.

4. Discussion

The mandatory mass vaccination campaign, which started in 2008 with inactivated BTV-8 vaccines in Germany, contributed significantly to the eradication of the virus [2]. In 2009, only cattle with very low BTV-genome loads in the blood could be detected in the first six months of the year. These weak PCR positive samples can be interpreted as the confirmation of old infections within the year 2008. No "fresh" BTV infection could be detected in 2009 in Germany and the following years. In 2010, the vaccination program switched to a voluntary campaign. On February 15th, 2012,

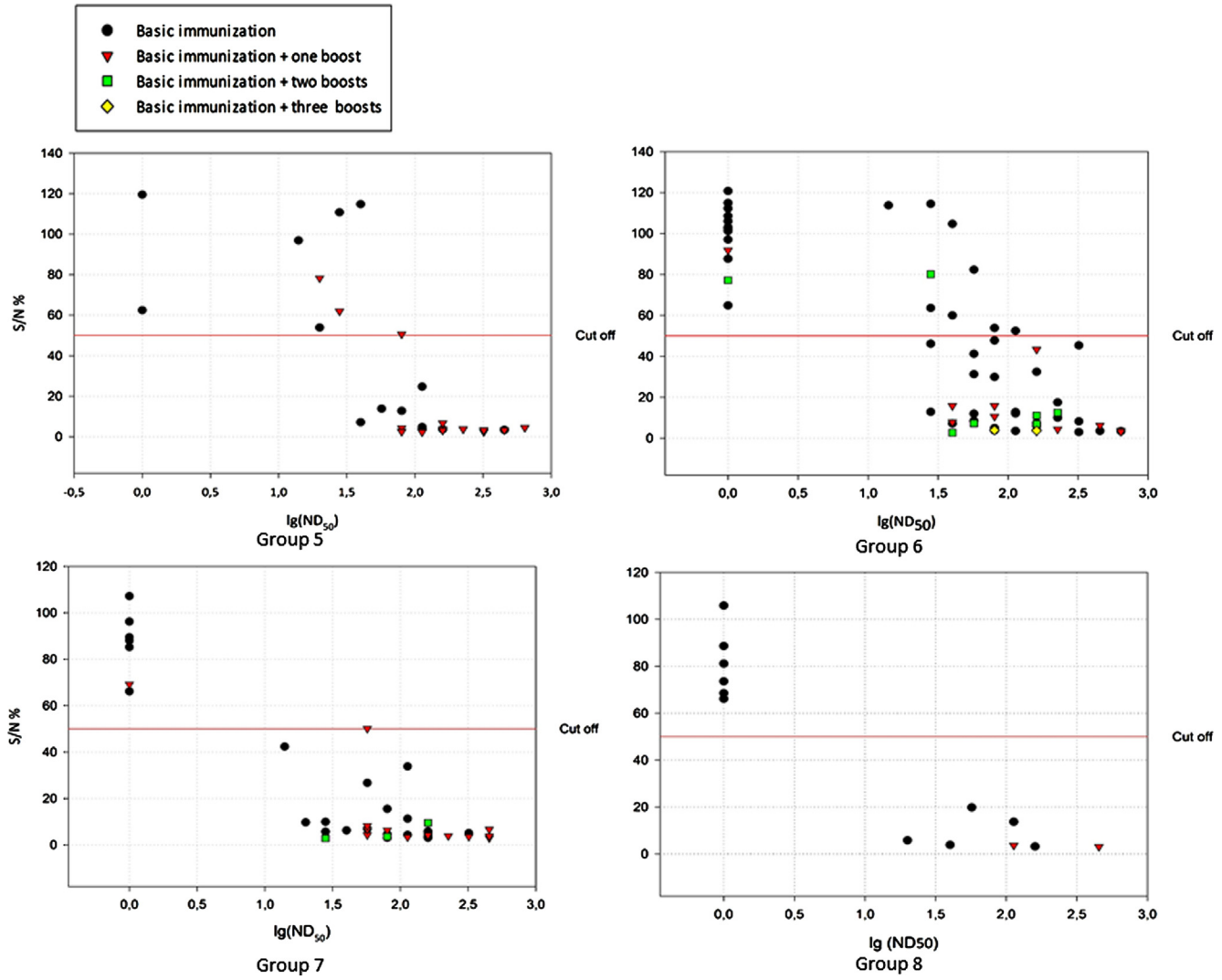

Fig. 1. Serum samples sorted by the group (5–8) and the number of vaccinations (1–4). The y-scale shows the ELISA results. The cut-off line divides the ELISA results in positive samples with less than 50% S/N and negative samples with more than 50% S/N value. The x-scale shows the SNT results. Samples with a $\log(ND_{50}) \geq 1$ are considered as positive, negative samples in SNT are shown with value 0. *Number of vaccinations: see legend of Table 1.

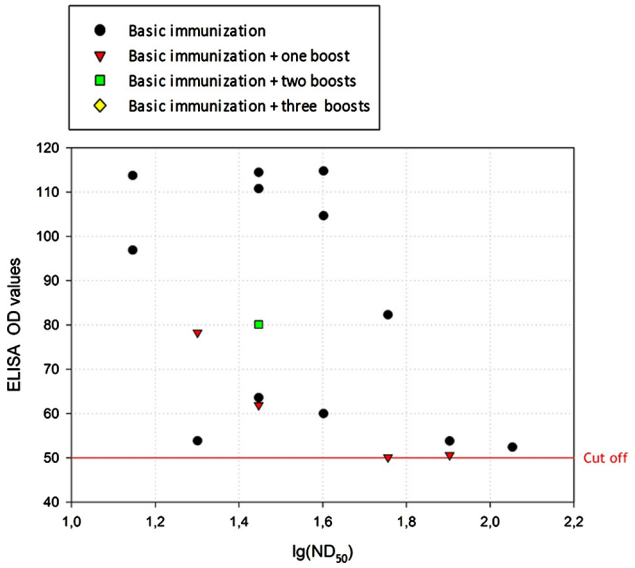

Fig. 2. Serum samples negative in the cELISA, but positive in the BTV-8-SNT sorted by the number of vaccinations. Six cattle with the basic immunization reacted positive in the BTV-8-SNT but strongly negative in cELISA. The y-scale shows the ELISA results. The cut-off line divides the ELISA results in positive samples with less than 50% S/N and negative samples with more than 50% S/N value. The x-scale shows the SNT results. Samples with a $\log(ND_{50}) \geq 1$ are considered as positive, negative samples in SNT are shown with value 0. *Number of vaccinations: see legend of Table 1.

Germany declared itself free from BTV [2] and BTV-8 vaccination was stopped completely for several years.

The mandatory and voluntary BTV-8 vaccination campaign over the years 2008 to 2012, the obligatory registration of BTV vaccinations in an official database, and the absence of "fresh" outbreaks of BTV since 2009 in Germany enabled us to the investigate the long-term humoral response after BTV-8 vaccination in older cattle. We investigated the antibody longevity in cattle for up to eight years post BTV-8 vaccination and the influence of booster vaccinations during this time. Two different serological methods (cELISA and SNT) were used for the investigations. Seven years post BTV-8 vaccination 83.7% of the analysed cattle were seropositive in both the cELISA and the SNT, and eight years post BTV-8 vaccination still more than 50% were seropositive. So far, antibody longevity of BTV-8 antibodies has been described for up to six years post vaccination [9,10]. Our results of BTV-8 seropositive cattle for five and six years post BTV-8 vaccination (93.8% and 79.4%) are comparable to the published seroprevalences for these time windows [9,10]. Less than 20% of the vaccinated cattle were seronegative for BTV even after several years. The existence of non- or poor responders after vaccination is a well-described phenomenon for several viral vaccines including BTV vaccines [12,13]. Differences in the immune response in cattle as shown for different booster vaccines [14], but also the training of veterinarians in vaccine application could influence the vaccination success [15]. Furthermore, the antibody responses to vaccination are modified by environmental and host genetic factors [16].

As there was not a single serum positive in the cELISA and negative in the BTV-8-SNT, the specificity of the used cELISA was 100%. The mismatches of the used serological methods were solely based on negative cELISA and positive SNT results, which was reported in previous serological studies as well [6]. The two serological methods target different BTV antibodies. VP7 as the major determinant of serogroup specificity is used in the cELISA, whereas the SNT detects with a high sensitivity antibodies against the serotype specifying VP2. A certain booster effect after re-vaccination could be observed, since the percentage of seropositive animals increased with the number of vaccinations. Cattle with only the basic immunization achieved a seroprevalence of 72.8%, whereas in cattle with one to three boost vaccinations, seropositivity increased to >90%. This statistically significant data support the efficiency of the booster vaccination as reported before [5].

However, the performed study does not provide information on the development of antibody titres over time in individual animals and is not able to show differences in the efficiencies of the commercially available BTV-8 vaccines. The selected bovine samples from older cattle do not reflect the current BTV-8 seroprevalence in Germany. As no challenge experiments were done, no statement with regard to the status of protection in cattle can be made, even though, a strong correlation between seropositivity and protection has been described previously [17–19]. This fact is supported by the latest findings from BTV-8 re-emergence in France in 2015, where seropositive cattle did not show a BTV-8 infection [10].

The results of our study show the huge power of inactivated BTV-8 vaccines and their long-term benefit, as antibodies were still detectable up to eight years after the last BTV-8 vaccination. All serology-based BTV screening programs are influenced by these long persisting vaccine-induced antibodies. This has to be taken into account by diagnosticians and epidemiologists.

Table 2
Overview of seronegative bovine serum samples in the cELISA and the BTV-8 SNT sorted by their group (group 5–8 represents the approximated time 5–8 years between the last BTV-8 vaccination and time of sampling), and the number of vaccinations.

	Seronegative cattle based on different numbers of vaccination*				Seronegative cattle depending of the groups	
Group°	1	2	3	4	Number	Percentage
5	2	0	0	0	2 of 32	6.25%
6	11	2	1	0	14 of 68	20.59%
7	6	1	0	0	7 of 43	16.28%
8	6	0	0	0	6 of 14	42.86%
5–8	25 of 92	3 of 53	1 of 10	0 of 2	29 of 157	18.47%
Percentage	27.17%	5.66%	10.00%	0%	18.47%	

° Groups 5–8: see legend of Table 1.
* Number of vaccinations: see legend of Table 2.

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Authors' contribution

CR participated in design of the study, collected, interpreted the data and wrote the manuscript. BH designed the study, interpreted the data, wrote the manuscript and participated in revision of the manuscript. MB contributed to the study design with his expert knowledge on bovine vaccinology, in data interpretation and participated in the revision of the manuscript.

Declaration of interest

The authors have no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.082.

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