Veterinary Microbiology 160 (2012) 35–42

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Cross-sectional study of bluetongue virus serotype 8 infection in South American camelids in Germany (2008/2009)

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ARTICLE INFO

Article history:
Received 4 February 2012
Received in revised form 10 May 2012
Accepted 18 May 2012

Keywords:
Bluetongue virus
BTV-8
South American camelids
Llama
Alpaca
Germany

ABSTRACT

Bluetongue (BT) is a major disease of ruminant livestock that can have a substantial impact on income and animal welfare. In South American camelids (SAC), fatalities related to bluetongue virus (BTV) infection were reported in Germany and France during the recent BTV-8 and BTV-1 epizootics, which raised concern about the role of SAC in the epidemiology of BTV.

Therefore, a large-scale serological and virological study was conducted in Germany from autumn 2008 to spring 2009. Risk factors associated with BTV infection were analysed by multiple logistic regression. These included age, species, gender and housing arrangements of SAC as well as the location of the herds and the presence of ruminants on farms.Altogether, 249 (14.3%) of 1742 SAC were found seropositive by BTV ELISA, and 43 (47.3%) of the 91 herds had at least one BTV-seropositive SAC. However, no BTV RNA was detected in any of the seropositive samples. Seroprevalence depended on the sampling region and probably on age, but not on any other analysed risk factors associated with BTV infection in ruminants. The highest seroprevalence was found in the west of Germany where the BTV-8 epizootic started in 2006. Recorded BTV-8 related disease and fatalities are discussed. Although the prevalence of BTV-8 antibodies was high in some regions, the virological results indicate that SAC play a negligible role in the epidemiology of this virus infection.

1. Introduction

Bluetongue (BT) is an infectious, non-contagious, haemorrhagic disease of ruminants (reviewed by MacLachlan et al., 2009). It is caused by bluetongue virus (BTV), an orbivirus within the family *Reoviridae* that is transmitted by biting midges of the *Culicoides* genus (Diptera, Ceratopogonidae). In August 2006, BTV-8 was the first-ever BTV serotype detected in Northern Europe. Since then, the infection has spread throughout Europe, and probably has caused greater economic damage than any previous single-serotype BTV outbreak before (Wilson and Mellor, 2009). Compulsory vaccination of domestic ruminants was successfully implemented in Germany from 2008 to 2009 (BTV-8) and in other European countries to contain the BTV-8 and BTV-1 epizootics (Wilson and Mellor, 2009; Gethmann et al., 2010). BTV vaccination of SAC has generally not been mandatory in Europe, but seroconversion to vaccination with inactivated BTV-8 vaccines was recorded in most SAC depending on the

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http://dx.doi.org/10.1016/j.vetmic.2012.05.028

Table 1
Number of SAC per herd tested for BTV-8 antibodies in Germany 2008/2009, number of farms on which sample sizes were lower than required assuming a 5% prevalence with 95% probability (Cannon and Roe, 1982) and median no. of missing samples per herd.

No. of tested SAC per herd	No. of examined herds	Proportion of herds (%)	No. of herds with lower sample size	Median no. of missing samples per herd
<8	7	7.7	1	1
8–10	18	19.8	4	1
11–20	41	45.1	25	2
21–40	14	15.4	6	13
41–60	11	12.1	0	–
Total	91	100	36	2

vaccine dose (Zanolari et al., 2010; Schulz et al., submitted for publication).

Recent studies have indicated that wild ruminants and Old World camelids can play a role as reservoirs leading to the dissemination and persistence of BTV and an increased risk of BTV infection in domestic ruminants (Falconi et al., 2011; García-Bocanegra et al., 2011; Batten et al., 2011).

In ruminants, BTV infection and manifestation of BT disease depends on species, breed, virus strain, individual resistance, age and sex (Ward et al., 1994; Brodie et al., 1998; MacLachlan et al., 2009; Linden et al., 2010; García-Bocanegra et al., 2011).

In South American camelids (SAC), serological evidence for natural BTV infection was documented in Peru (Rivera et al., 1987) and the USA (Mattson, 1994; Fowler, 1998). During the recent BTV-8 and BTV-1 epizootics in Europe, BTV RNA and antibodies were found in several SAC in Germany and France (Henrich et al., 2007; Meyer et al., 2009) but not in a serological survey in Switzerland (Zanolari et al., 2010).

Before 2007, SAC were considered resistant to BT disease (Rivera et al., 1987; Mattson, 1994; Afshar et al., 1995). However, recent reports of sporadic fatal cases and the detection of BTV RNA in SAC raised concern about their role in BTV epidemiology (Henrich et al., 2007; Meyer et al., 2009; Ortega et al., 2010). The potentially devastating consequences of BT together with the recent BTV-related fatalities prompted the investigation of BTV infection in SAC. A large-scale serological and virological study was conducted in Germany. Risk factors influencing BTV infection in ruminants were evaluated for SAC by multiple logistic regression.

2. Materials and methods

2.1. Study design and sampling

The study population was selected by multi-stage sampling. Among a total of 227 contacted farms in Germany, most ($n = 170$) had been systematically selected by postal code from a mailing list of an association of breeders, owners and friends of SAC[2] (NWK e.V.) from summer 2008 to spring 2009. The other 57 owners were contacted at meetings of other SAC associations and after recommendation by other participants. All owners with at least eight SAC that agreed to participate in the cross-sectional study ($n = 91$) were visited once, and individual SAC were randomly selected. Three farms that kept less than eight SAC were also accepted in the study as they were located in regions were only a few herds were available.

For sample size calculations a BTV prevalence of 5% was assumed (Conraths et al., 2009). When the study was designed, no exact data on the number of SAC in Germany were available. Based on an estimate of 5000 SAC in 2004 (Rohbeck, 2006), a current population size of 10,000 animals was assumed. The overall sample size necessary to detect a 5% BTV (sero-)prevalence (Conraths et al., 2009) with 95% probability and an accepted error of 1% was calculated (Cannon and Roe, 1982) to be at least 1544. The number of blood samples to take per farm was calculated from the size of the SAC herd. A maximum sample size of 59 for large herds was required to detect at least one serologically or virologically BTV-positive animal in a herd with a 95% probability (Cannon and Roe, 1982). See Table 1 for the number of SAC tested per herd.

Blood samples were taken from 1742 (67% of 2601) unvaccinated SAC (1249 alpacas, 479 llamas, 14 SAC of other breeds) on 91 farms located in all federal states of Germany, except city states (Fig. 1, Table 2; and Table 1 in the supplemental material). In general, non-weaned crias from sampled dams also participated in the study. All samples were taken between mid-September 2008 and early May 2009. Most herds (91%) were visited from November 2008 to March 2009, outside the main BTV vector season.

2.2. Serology

Blood was drawn by jugular puncture and collected in plain tubes and tubes with potassium EDTA. Serum was harvested and stored at −20 °C, while EDTA-treated blood was stored at 4 °C until further analysis. All sera were analysed in the commercially available serogroup-specific, but not species-specific PrioCHECK® double recognition (DR) ELISA (INGEZIM BTV DR 12.BTV.K0, INGENASA, Madrid, Spain[3] or PrioCHECK® BTV DR, Prionics Deutschland GmbH,

[2] Verein der Züchter, Halter und Freunde von Neuweltkameliden e.V., http://www.lamas-alpakas.de.

[3] The INGEZIM BTV DR test was developed by Ingenasa and is distributed in Germany by Prionics GmbH under their own name. The products are identical and in the text, the PrioCHECK® BTV DR label is used for both.