
P. Zanolari, V. Chaignat, C. Kaufmann, M. Mudry, C. Griot, B. Thuer, and M. Meylan

Background: Outbreak of bluetongue virus serotype-8 (BTV-8) infection in domestic ruminants in Northern Europe.

Objective: To investigate the South American camelids’ (SAC) susceptibility to BTV-8 infection, their role in the epidemiology of the disease, and the use of currently available serological screening tests in SAC in an endemic region.

Animals: Three hundred and fifty-four unvaccinated and 27 vaccinated SAC (170 llamas, 201 alpacas), ranging in age from 1 month to 17 years between June and August 2008. The SAC originated from 44 herds throughout the country, representing 10% of the Swiss SAC population.

Methods: Prospective, observational study of a convenience sample of SAC. Serum samples were analyzed with 2 serological screening tests. When results diverged, a 3rd ELISA was carried out for confirmation (ID Screen Bluetongue Competition ELISA kit).

Results: All sera from the 354 unvaccinated animals were negative in the endemic region. Reliable seroconversion was observed after administration of 2 doses of vaccine.

Conclusions and Clinical Importance: This study suggests a low susceptibility of SAC to BTV-8 despite the presence of the virus in the cattle and small ruminant population, indicating that SAC do not play a major role in the epidemiology of BTV-8. Furthermore, these results indicate that commercially available serological tests for BTV-8 can be used in SAC.

Key words: Alpaca; ELISA; Infectious disease; Llama; Orbivirus.

Bluetongue (BT) is a noncontagious viral disease affecting domestic and wild ruminants and is transmitted by biting midges (Culicoides spp.).1–7 The bluetongue virus serotype-8 (BTV-8) first appeared in the Netherlands, Belgium, and Germany in the summer of 2006 and spread to surrounding countries, reaching Switzerland by the end of October 2007.8–11 This serotype had not previously been isolated in Northwestern Europe and resulted in severe clinical signs in sheep and cattle.12–14 By the end of May 2008, BTV-8 had been identified in cattle and sheep from 6 farms in Switzerland.

Although South American camelids (SAC) (Artiodactyla/ Tylopoda) are phylogenetically related to domestic and wild ruminants (Artiodactyla/ Ruminantia), they appear only moderately susceptible to BTV infection. In 1 case report, infection in a pregnant llama led to dyspnea followed by abortion.15 Although no virus was isolated, paired serum samples showed seroconversion with a high antibody titer to BTV. There are only a small number of reports on BTV seroprevalence in SAC, and the results vary widely. In Peru, 24 of 114 (21%) alpacas were seropositive,16 but in Oregon, USA, only 4 of 270 (1.5%) llamas were seropositive for BTV.17 No seropositive animals were detected in 390 llamas tested in Argentina.18 Despite the high pressure of infection in continental Europe since 2006, clinical disease related to BTV infection has been reported in only 3 SAC. A female alpaca from a farm in Germany had peracute disease with dyspnea, anorexia, recumbency and lethargy, and subsequently died. Polymerase chain reaction showed BTV sequences in blood, lymph node, and spleen samples, but the serotype was not determined.19 In France, BTV serotype-1 was isolated in 2 llama herds: in 1 herd, all llamas were seropositive but none had clinical signs of disease; in the other, 1 animal aborted and 2 llamas died of acute BTV-1 infection. Both llamas had acute severe dyspnea and died within 24 hours of the onset of clinical signs.20 To date, BTV-1 has not been isolated in Switzerland.

Because vector control with insecticides was deemed insufficient to control the spread of BTV-8 in the short to intermediate term, most European countries undertook widespread vaccination of susceptible species with the newly developed BTV-8 specific vaccines in 2008. In Switzerland, vaccination against BTV-8 of all cattle, sheep, and goats older than 3 months was mandatory,21 but vaccination of SAC was not.

There are currently no known cases of clinical diseases because of BTV-8 infection in SAC in Switzerland. The goal of the present study was to determine whether SAC incurred subclinical infection during the outbreak of BTV-8 and might thereby have played in propagating the disease.

Abbreviations:

BT bluetongue
BTV bluetongue virus
IgM immunoglobulin M
SAC South American camelids
VP virus protein
Materials and Methods

In May 2008, 350 members of the Association of Llama and Alpaca breeders of Switzerland and of the Swiss Alpaca Association were contacted regarding the use of their animals to determine the seroprevalence of BTV-8. Of 100 owners who responded, 44 were randomly chosen to participate in the study.

The age, sex, and species of the animals as well as possible contact with other even-toed species on the farm were recorded. Blood was collected from the jugular vein of 354 animals originating from 38 unvaccinated herds from June to August 2008. Blood samples were refrigerated in an insulated container and centrifuged within 4 hours of collection. The serum was harvested and stored at −20°C until further analysis.

A total of 27 animals in 6 of the 44 herds had been vaccinated subcutaneously with Bovilis BTV-8a by the time of the start of the study because the owners had been concerned about the rapid spread of the disease in Europe. Animals from 3 of the 6 herds had been vaccinated twice, 21 days apart, and those from the other 3 herds had been vaccinated once. Serum samples were also collected from these 27 vaccinated animals in order to investigate seroconversion.

The statistic software program WinEpiscope 2.0b was used to determine that a minimum of 300 blood samples were required to detect at least 1 positive animal, assuming a BTV-8 seroprevalence in Switzerland of 1%. At the time of the study, the number of selected herds as well as individual animals represented at least 10% of the total number of herds and the total population of SAC in Switzerland.

All sera were tested for antibodies with 2 commercial test kits: the Bluetongue Virus Antibody Test Kitc and the INGEZIM BTV DR Test.d Samples with diverging results were additionally tested with the ID Screen Bluetongue Competition ELISA Kit®. All tests were carried out in duplicate and according to the manufacturers’ specifications. The Bluetongue Virus Antibody Test Kit and ID Screen Bluetongue Competition ELISA Kit are competitive ELISAs (cELISA), which can be used for identification of BTV antibodies in all species of animals. The test plates are coated with BTV virus protein 7 (VP 7) antigen (Ag), which binds the antibody to VP 7 Ag independently of the species. In positive serum samples, the BTV antibody binds to the Ag on the plate, thus preventing (the commercial antibody enzyme marker) from binding, this being applied last. Positive serum samples therefore yield a colorless reaction. The INGEZIM BTV DR Test ELISA is based on the principle of double recognition and can also be used in all animal species. The plates are coated with BTV VP 7 Ag, which binds the antibody to VP 7 Ag from all species. After rinsing, a VP 7 Ag linked to an enzyme marker is applied, which binds to free binding sites of the already bound immunoglobin from the sample (maximum 1 binding site for IgG, maximum 9 binding sites for IgM). Positive serum samples yield a color reaction. In cattle, this test combination has an estimated sensitivity of 99% and a specificity of 99–100% 14 days after infection (validation data not shown). No specific data for SAC are available to date.

Results

The distribution of the 38 farms from which the 354 serum samples (159 llamas and 195 alpacas) were collected throughout Switzerland is shown in Figure 1. Each farm had between 2 and 141 animals and the number of samples collected per farm ranged from 2 to 26. There were 150 males (83 intact and 67 castrated) and 204 females ranging in age from 1 month to 17 years. Only 20 animals were identified as adults without an exact indication of age. The mean age of the other 334 animals was 4.8 ± 3.2 years. Of the 354 animals, 336 were older than 1 year of age and therefore could have been infected with BTV-8 in the fall of 2007. Thirteen of the 38 farms had no animals other than SAC. Of the remaining 25 farms (66% of farms and 70% of tested animals), 11 had cattle, 8 had sheep, 8 had goats, 1 had zebu, 1 had camels, 4 had pigs, 6 had horses, 1 had ponies, 5 had donkeys, and 5 had fowl. The location of the farms used in the present study in relation to the location of BTV-8 infection in cattle and sheep in Switzerland, which occurred shortly before the start of this study, as well as the current situation until February 2009 is shown in Figure 1.

All serum samples from the 354 unvaccinated animals yielded negative test results for the Bluetongue Virus Antibody Test Kit and the INGEZIM BTV DR Test.

Of the 27 animals vaccinated against BTV-8 infection, 14 had positive screening ELISA results (Bluetongue Virus Antibody Test Kit and INGEZIM BTV DR Test), and 3 had positive Bluetongue Virus Antibody Test Kit but negative INGEZIM BTV DR Test results (of these 1
was positive in the ID Screen Bluetongue Competition ELISA Kit test and 2 gave inconclusive results). These 3 animals had only been vaccinated once. One serum sample was positive in the INGEZIM BTV DR Test and negative in the Bluetongue Virus Antibody Test Kit. The remaining 9 samples from vaccinated animals all had negative ELISA tests (Bluetongue Virus Antibody Test Kit and INGEZIM BTV DR Test); 6 of these animals had been vaccinated 1 week before blood collection only and the other 3 had received only 1 vaccination.

Discussion

Cattle, sheep, goats, wild ruminants, SAC, exotic herbivores, and carnivores are susceptible to BTV infection.\(^4,7,9,11,19,20,22-25\) To date, death attributable to BTV infection in SAC has been reported in 1 alpaca and 2 llamas only.\(^19,20\) Previous reports have shown that SAC can be infected with BTV and produce antibodies.\(^16-20\) However, because no experimental infection studies are available, the degree of susceptibility of SAC to BTV infection and the degree of protection afforded by antibodies is still unknown. In the present study, it was assumed that the number of SAC with antibodies to BTV in Switzerland would be very small. Therefore, serum samples collected in the present study represented at least 10% of the total Swiss population of SAC, including llamas and alpacas, being collected from all regions of Switzerland. For a reliable estimate of seroprevalence, 2 antibody ELISAs with different testing principles were used as screening tests. The competitive Bluetongue Virus Antibody Test Kit ELISA detects antibodies produced some time before blood collection (2–3 and more weeks before analysis), and should therefore have identified animals infected during the outbreak of BTV-8 in Switzerland in the fall of 2007. In cattle and sheep, the Bluetongue Virus Antibody Test Kit ELISA reliably detects antibodies for a minimum of 5 years after infection (K. Nomikou, personal communication). The INGEZIM BTV DR Test ELISA is the test of choice for detecting antibodies (IgM) shortly after infection (6–8 days postinfection in experimentally infected sheep, at a time when other BTV ELISAs were negative) (G. Worwa et al, unpublished data). In the present study, blood samples were collected from June to August 2008 when the activity of biting midges started to intensify. Thus, SAC infected shortly before June 2008 would have been identified by this test. The basic principle of the cELISA has already been used to detect BTV antibodies in wild ruminants and llamas.\(^26\) Indeed, we have observed that SAC produce BTV antibodies that can be detected by a cELISA after immunization with commercially available (inactivated) vaccines, Bovilis BTV-8 or BTVPUR AlSap8 (P. Zanolari et al, unpublished data). In the present study, animals that had been vaccinated twice were all, except for one of them, seropositive in at least 1 of the screening ELISA tests. Therefore, if seroconversion occurs after vaccination with inactivated vaccine in SAC, it can be assumed that it will also occur after natural infection.

Despite the high pressure of infection in domestic ruminants in France and Germany,\(^27,28\) there have been only 2 reports of clinical BTV infection in SAC.\(^19,20\) The reason therefore may be that the course of BTV-8 infection in SAC is mainly subclinical, similar to that observed in goats.\(^29,30\) The official Swiss register of BTV-8 infection in cattle and sheep confirmed that cases have occurred in regions inhabited by the SAC used in our study (Fig 1). However, our results indicate that subclinical infection of SAC did not occur despite the virus being present in the ruminant population.

In Switzerland, BTV-8 infection was recorded in 4 cattle herds in 2007 and in 31 cattle herds and 12 sheep flocks in 2008. A related Toggenburg orbivirus was identified in 2 goat herds.\(^6\) To date, 12 cattle farms have been affected with BTV-8 in 2009. The majority of cases were subclinical and overall only 24 animals showed overt signs of BTV-8 infection. As most infections were subclinical, it must be assumed that more herds were infected, which were not identified as such because of lack of disease clinical signs. Indeed, most registered cases were diagnosed through analyses prescribed for the export of animals or within an official surveillance program. The number of reported cases, therefore, is not likely to adequately reflect the actual pressure of infection. It is important to note that the vaccination against BTV-8 of cattle, sheep, and goats older than 3 months and not kept on an alpine community pasture (>1,000 m above sea level) was mandatory as of June 2008 in Switzerland. Vaccination could not begin before June because BTV-8 vaccines were not available in sufficient quantities in Europe. After the introduction of the mandatory vaccination program, routine periodic serological monitoring of cattle and sheep became obsolete. Only animals that were imported into or exported from Switzerland, or that were suspected of being infected were tested for BTV. Extrapolation from data of BTV infection outbreaks in other regions of Europe during 2007/2008 suggests that the pressure of infection on the susceptible animal population in Switzerland was considerable. In the Netherlands, the seroprevalence of BTV-8 in 2006 was 0% in goats and 7% in sheep, but it increased sharply to 47% in goats and 70% in sheep 1 year later.\(^31\) Among unvaccinated animals, the disease appeared to spread rapidly after isolated outbreaks in Western Europe. A similar situation with a rapid spread of infection also occurred during the massive outbreak of BTV-8 in neighbouring regions of France, where only part of the animal population had been vaccinated (http://ec.europa.eu/food/committees/regulatory/sfccah/animal_health/presentations/bt_0102042009_fr.pdf). Immunization against BTV-8 in Switzerland was started in the summer of 2008 shortly before the height of activity of Culicoides spp. The presence of infective biting midges during the ensuing summer was therefore almost certain, especially in the regions of Switzerland located close to France. After feeding on viraemic animals in these regions, infected midges could have been carried eastward by the wind,\(^32\) which may explain the relatively high number of infected cattle and sheep in the western parts of Switzerland. Despite the high pressure of infection, no
seropositive SAC were identified in those regions. Ruminants other than SAC were kept in 66% of the herds included in the study. As Culicoides spp. are attracted by these animals (more than by SAC),33 this might result in higher numbers of midges present on such farms than in herds comprising SAC only.

That no animals with detectable levels of antibodies against BTV-8 were identified in a serological survey of at least 10% of the Swiss SAC population suggests that these animals are either only moderately susceptible to infection with this BTV serotype and/or that the virus does not replicate efficiently within these hosts. Alternatively, SAC may not commonly be bitten by Culicoides spp. Furthermore, the virus levels in the population have most likely decreased since the mandatory vaccination of domestic ruminants was introduced in Switzerland. In contrast, a seroprevalence of 21% was detected in alpacas in Peru, but this discrepancy may be associated with different species of Culicoides living in different regions, microclimates and altitudes (F. Schaffner, personal communication), which may be more or less efficient in the transmission of BTV.34–36 It is therefore possible that Culicoides obsoletus and Culicoides pulicaris, which have been associated with BTV transmission in Europe but not in South America, have a different host range in different regions. Midge of the obsoletus-complex, which are considered to be mostly responsible for the transmission of BTV in Central Europe, were predominant below 1500 m above sea level in Switzerland. With increasing altitude, midges of the pulicaris-complex prevailed. No populated area in Switzerland was free of midges.37

Footnotes

a Bovis BTV8, Intervet, Boxmeer, the Netherlands

References


