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Research paper

Culicoides vector species on three South American camelid farms seropositive for bluetongue virus serotype 8 in Germany 2008/2009

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ABSTRACT

Palearctic species of *Culicoides* (Diptera, Ceratopogonidae), in particular of the Obsoletus and Pulicaris complexes, were identified as putative vectors of bluetongue virus serotype 8 (BTV-8) on ruminant farms during the epizootic in Germany from 2006 to 2009. BTV may cause severe morbidity and mortality in ruminants and sporadically in South American camelids (SAC). However, the fauna of *Culicoides* spp. on SAC farms has not been investigated.

Therefore, the ceratopogonid fauna was monitored on three farms with BTV-seropositive SAC in Germany. Black–light traps were set up on pastures and in stables from summer 2008 to autumn 2009. Additionally, ceratopogonids were caught in emergence traps mounted on llama dung and dung-free pasture from spring to autumn 2009. After morphological identification, selected *Culicoides* samples were analysed for BTV-RNA by real-time RT-PCR. The effects of the variables 'location', 'temperature' and 'humidity' on the number of *Culicoides* caught in black–light traps were modelled using multivariable Poisson regression.

In total, 26 species of *Culicoides* and six other genera of biting midges were identified. The most abundant *Culicoides* spp. collected both outdoors and indoors with black-light traps belonged to the Obsoletus (77.4%) and Pulicaris (16.0%) complexes. The number of *Culicoides* peaked in summer, while no biting midges were caught during the winter months. Daily collections of *Culicoides* were mainly influenced by the location and depended on the interaction of temperature and humidity. In the emergence traps, species of the Obsoletus complex predominated the collections.

In summary, the absence of BTV-RNA in any of the analysed *Culicoides* midges and in the BTV-seropositive SAC on the three farms together with the differences in the pathogenesis of BTV-8 in SAC compared to ruminants suggests a negligible role of SAC in the spread of the virus. Although SAC farms may provide similar suitable habitats for putative *Culicoides* vectors than ruminant farms, the results suggest that geographic and meteorological factors had a stronger influence on *Culicoides* abundance than the animal species.

1. Introduction

Bluetongue disease is notifiable to the OIE (World Organization for Animal Health) since it may cause severe morbidity and mortality in domestic ruminants and has a substantial economic impact in affected countries. Palearctic *Culicoides* spp. belonging to the Obsoletus and Pulicaris complexes as well as *Culicoides dewulfi* were incriminated as the main vectors of bluetongue virus serotype 8 (BTV-8) in ruminant herds during the recent epizootic of BTV-8

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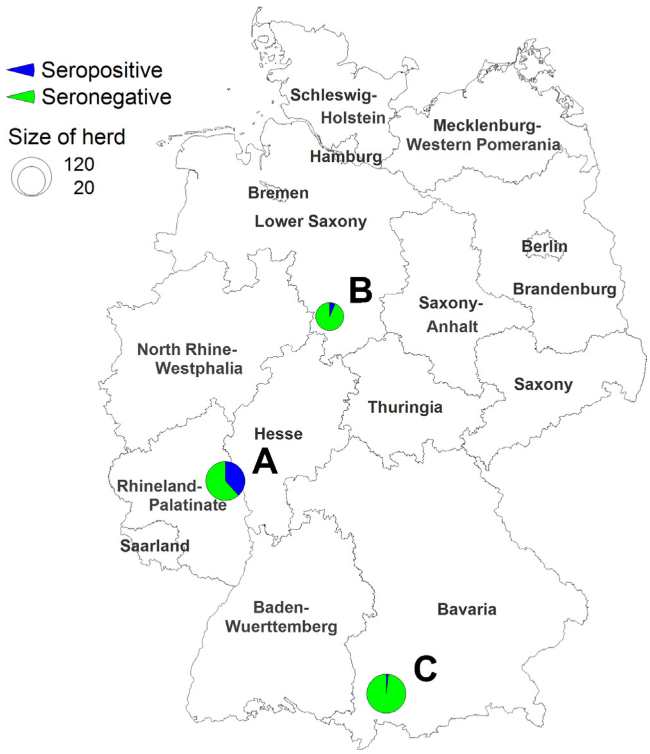

Fig. 1. Map showing locations of the black-light traps set up on three SAC farms in Germany 2008/2009. Dark blue wedges show proportions of seropositive SAC. The highest BTV seroprevalence in SAC was found on the farm in Hesse (farm A).

in Northern and Central Europe that started in 2006 (Meiswinkel et al., 2008; Hoffmann et al., 2009; Eschbaumer et al., 2010). In Germany, BTV-8 was eventually eradicated by mandatory vaccination of domestic ruminants, and no BTV re-emergence has been registered since 2009 (Gethmann et al., 2010; FLI, 2012). As of 15 February 2012, Germany and the Benelux countries were declared free of BTV (LAVES, 2012). However, BTV-6 and BTV-11 temporarily emerged to northern Europe and three novel BTV serotypes (BTV-25, -26 and -27) have been discovered since 2008 (Hofmann et al., 2008; Eschbaumer et al., 2010; Maan et al., 2011; Zientara et al., 2014). Furthermore, the novel teratogenic Schmallenberg virus (SBV), a Simbu virus belonging to the *Orthobunyavirus* genus, has recently been detected in ruminants and in Palearctic *Culicoides* spp. of the Obsoletus and Pulicaris complexes, *C. dewulfi* and *Culicoides nubeculosus* in northern Europe (Hoffmann et al., 2012; Rasmussen et al., 2012; Elbers et al., 2013; Larska et al., 2013; Balenghien et al., 2014; Wernike et al., 2014). The emergence of vectorborne viruses is obviously not predictable (Carpenter et al., 2009; Balenghien et al., 2014). Hence, investigation of factors influencing abundance of arbovirus vectors is crucial to be able to predict the epidemiology and spread of an emerging vectorborne disease, and to implement appropriate control measures (Purse et al., 2005; Werner et al., 2012).

South American camelids (SAC) are also susceptible to BTV and SBV infection (Schulz et al., submitted for pulication; Jack et al., 2012; Schulz et al., 2012a,b). German field studies of BTV-8 and SBV infection in SAC revealed a high proportion of SAC that tested positive for antibodies against BTV (14.3% of 1742) and SBV (62.4% of 502) (Schulz et al., submitted for pulication; Schulz et al., 2012a,b). However, little is known about *Culicoides* spp. and other ceratopogonids occurring on SAC farms. A few Obsoletus complex midges were collected with an emergence trap mounted on llama dung (Werner et al., 2012), indicating that putative vectors of BTV and SBV may occur on SAC farms and use SAC dung as breeding habitat.

To more profoundly scrutinize the role of SAC in the spread of *Culicoides*-borne viruses, this study aimed at monitoring the diversity and possible breeding habitats of *Culicoides* spp. and other ceratopogonids with emphasis on vectors of BTV-8 on three SAC farms during the BTV-8 epizootic in 2008. Potential environmental key factors that may influence *Culicoides* abundance and BTV prevalence on ruminant farms were statistically analysed for the SAC farms (Mellor et al., 2000; Purse et al., 2012).

2. Material and methods

2.1. Trap locations

The population dynamics of ceratopogonids was monitored on three German SAC farms with BTV-seropositive (Table 1) llamas (*Lama glama*) and alpacas (*Vicugna pacos*) that participated in a field study of BTV-infection in SAC (Schulz et al., 2012b). The farms were selected prior to testing the BTV infection status of the SAC herds. They were located in areas where BTV prevalence in ruminants was either relatively high (southern Hesse and southern Lower Saxony in Central Germany) or relatively low (Allgäu region in southern Bavaria, southeastern Germany) from 2006 to 2008 (Conraths et al., 2009). Assigned farm labels, trap locations and information on the landscape, animal husbandry and BTV-seroprevalence on the three SAC farms are presented in Fig. 1 and Table 1, respectively.

2.2. Trapping protocol

Biting midges were caught with black–light traps with a suction fan (BG-Sentinel^{TM} midge trap, Biogents, Regensburg, Germany) from July 2008 to June 2009 (farm A) or from July 2008 to November 2009 (farms B and C). On each farm, one trap was set up on pasture ("outdoors") and another one in the stable ("indoors"), at a height of about 1.5–2 meters. All traps were operated in the first seven consecutive nights of each month, activated by a photosensitivity switch, from dusk until dawn at a light intensity below 20 lx (Clausen et al., 2009; Hoffmann et al., 2009). From December 2008 to March 2009, outdoor traps were not operated since SAC were kept indoor at night, and *Culicoides* were not expected in outdoor traps due to the cold weather conditions (Wilson et al., 2007). Indoor traps were operated during the winter months to investigate the possibility of adult *Culicoides* overwintering in stables potentially warmed up by the animals' body temperature. During the respective catching period, biting midges were caught in catch beakers containing 70% ethanol and were harvested once a day. The collections were preserved in 70% ethanol until further analyses.

On farm A, an emergence trap (covered ground area: $100 \times 100\,cm^2$; www.fiebig-lehrmittel.de) was set up on fresh to several-day-old llama dung ('dung trap') from April to September 2009. SAC generally defecate and urinate on the same dung pile (Fowler, 2010). The selected dung pile had been used and regularly cleaned for around ten years. For comparison, a second emergence trap was set up on a dung-free area on the same pasture ('pasture trap') from May to September 2009. Eclosed biting midges attempting to escape through the tent roof of the emergence trap were caught in a catch beaker with 70% ethanol. Similar to the black–light traps, the emergence traps were operated in the first week of each month, but catch beakers were not replaced within these periods.

2.3. Morphological identification of ceratopogonids

In general, all collected ceratopogonids were sorted from the catches. From samples with exceedingly high *Culicoides* counts, a subsample of 500 individuals maximum was sorted, and the total number of *Culicoides* was estimated according to Van Ark and Meiswinkel (1992) to allow a reliable estimate on the collected