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Bluetongue and Epizootic Haemorrhagic Disease virus in local breeds of cattle in Kenya

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ABSTRACT

The presence of bluetongue virus (BTV) and Epizootic Haemorrhagic Disease virus (EHDV) in indigenous calves in western Kenya was investigated. Serum was analysed for BTV and EHDV antibodies. The population seroprevalences for BTV and EHDV for calves at 51 weeks of age were estimated to be 0.942 (95% CI 0.902–0.970) and 0.637 (95% CI 0.562–0.710), respectively, indicating high levels of circulating BTV and EHDV. The odds ratio of being positive for BTV if EHDV positive was estimated to be 2.57 (95% CI 1.37–4.76). When 99 calves were tested for BTV and EHDV RNA by real-time RT-PCR, 88.9% and 63.6% were positive, respectively. Comparison of the serology and real-time RT-PCR results revealed an unexpectedly large number of calves that were negative by serology but positive by real-time RT-PCR for EHDV. Eight samples positive for BTV RNA were serotyped using 24 serotype-specific real-time RT-PCR assays. Nine BTV serotypes were detected, indicating that the cattle were infected with a heterogeneous population of BTVs. The results show that BTV and EHDV are highly prevalent, with cattle being infected from an early age.

1. Introduction

Bluetongue virus (BTV) and Epizootic Haemorrhagic Disease virus (EHDV) are members of the genus Orbivirus, family Reoviridae (Mertens et al., 2005) and are transmitted by biting midges (*Culicoides* spp.). Bluetongue (BT) was first described in Africa in the early 19th century and can infect all species of ruminants, although clinical outbreaks are usually seen in susceptible European sheep breeds. There have been multiple incursions of BTV into Europe from Africa, the most serious caused by the strain of BTV serotype 8 (BTV-8). The outbreak spread across Europe between 2006 and 2009 and caused clinical signs in cattle, goats and sheep (EFSA Panel on Animal Health and Welfare, 2011). As limited sequence information is available for BTV strains circulating across Africa, it was not possible to conclude with certainty the origin of this virus, however full genome sequence analysis indicated that it may have originated from sub-Saharan Africa (Maan et al., 2008).

EHDV primarily infects deer, and cattle are thought to act as a reservoir. Outbreaks were reported in Morocco and Israel in 2006 and Turkey in 2007, where cattle exhibited mild clinical signs (Temizel et al., 2009; Yadin et al., 2008). Very little is known about the distribution of EHDV in Africa apart from the fact that EHDV-3 (now reclassified as EHDV-1) and EHDV-4 were isolated in Nigeria in the late 1960s and EHDV (serotype unknown) was isolated in South Africa in the 1990s (Savini et al., 2011).

The aim of this study was to improve current knowledge of the prevalence and distribution of EHDV and BTV in domestic cattle in sub-Saharan Africa. The study set out to estimate the seroprevalence of EHDV and BTV antibodies and the prevalence of infection (through the detection of viral RNA) and to identify the BTV and EHDV serotypes in a subset of samples from cattle in western Kenya.

2. Materials and methods

2.1. Study site

The samples analysed in this study were collected as part of the 'IDEAL' (Infectious Diseases in East African Livestock) project,

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which monitored infections in 548 indigenous calves, from birth to death or 12 months of age, in western Kenya, and is described in detail by Bronsvoort et al. (submitted). The field component of the study was carried out between October 2007 and September 2010, and the calves were located in households within 45 km of the town of Busia on the Kenya/Uganda border. The study area (Fig. 1) stretches from Lake Victoria in the south–west to the slopes of Mt. Elgon in the north–east and encompasses four Agro-Ecological Zones (AEZ): Lower Midlands (LM) 1, LM2, LM3 and Upper Midlands 3 (Jaetzold and Schimdt, 1983). The area has a warm and moist tropical climate with a bimodal rainfall pattern with two peaks (March to May and October to December), although there is moderate rainfall throughout year. Most of the area is cultivated but interspersed with wetlands covered with grassland and often used for communal grazing. The chief farming system is a small holder mixed crop/livestock system and the predominant breed of cattle is the small East African Zebu. Farmers also keep other livestock especially sheep and poultry. The calf selection was stratified by sublocation, which is the smallest administrative unit in Kenya, with the aim of recruiting the same number of calves per sublocation.

Calves were recruited during the first week of age, usually within the first 3–7 days after birth, and were routinely visited every 5 weeks until death or 51 weeks of age. Calf locations were geo-referenced using hand-held GPS devices (Garmin 12, Garmin Kansas, USA). The 5-week interval was chosen for logistical reasons to allow the field study to be completed within 3 years. The study was designed so that calf recruitment by sublocation was evenly distributed throughout the year. There is no planned breeding programme in these farms, with most animals being sired by an available bull. Some farmers made efforts to take their cows to specific bulls in the neighbourhood. The cattle grazed in communal grazing fields or were tethered in the household compound. Households which used stall-feeding were excluded from the study.

2.2. Sampling

At each 5-week visit the calves were clinically examined and biological samples were obtained for laboratory analysis. The samples used in this study were final visit samples, which were obtained from the calves at the last routine visit prior to the calf leaving the study or reaching 51 weeks of age. For serology, jugular vein blood was collected into plain Vacutainer™ (Becton Dickinson) tubes. After clotting, serum was recovered and aliquots were stored at −20 °C until use. Blood for RNA extraction was collected into EDTA-Vacutainer™ tubes, aliquotted into cryovials and stored at −20 °C. The samples were processed on the day of collection in a laboratory established by the project in Busia.

2.3. Serology

The detection of BTV-specific antibodies in serum was performed using a sandwich (double antigen) ELISA assay (ID-Screen Bluetongue Early detection ELISA, ID-Vet, France) according to the manufacturer's instructions. A blocking ELISA (LSIVET EHDV BLOCKING, Laboratoire Service International, Lyon, France) for the detection of EHDV-specific antibodies was used to test each sample. The assay was performed and analysed following the manufacturer's instructions.

2.4. Molecular analyses

2.4.1. RNA extraction

RNA was extracted from EDTA blood samples and known BTV and EHDV positive control samples using the Universal (Qiagen, Crawley, UK) extraction robot using the 'One for all' protocol.

2.4.2. Real-time RT-PCR

BTV RNA was detected by real-time RT-PCR using a modified version of a previously published protocol (Shaw et al., 2007), while EHDV RNA was detected using an ''in house'' EHDV specific real-time RT-PCR targeting genome segment 9 (unpublished).

2.5. Serotyping

BTV RNA was serotyped using 24 individual serotype-specific (segment 2) real-time RT-PCR assays (Mertens et al manuscript in preparation), while EHDV RNA was serotyped using 7 serotype specific gel-based RT-PCRs as described previously (Maan et al., 2010).

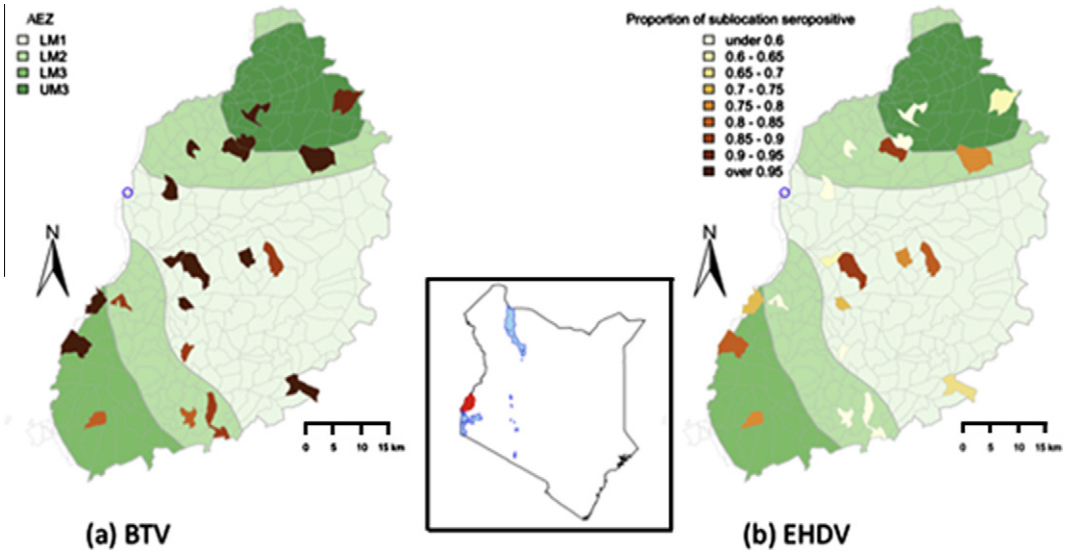

Fig. 1. Map of the study area showing the AEZs within the study area and the 20 sublocations from which calves were recruited. The distribution of calves seropositive at 51 weeks for BTV (a) and EHDV (b) is also shown. The inset map shows the location of the study area in western Kenya, and the circle indicates the location of the project laboratory in Busia.