Bluetongue in springboks and wildebeests in the Etosha National Park (Namibia): circulating strains and epidemiology.

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Bluetongue virus in springboks and wildebeests in the Etosha National Park (Namibia)

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Abstract

Bluetongue virus (BTV) is a double strand RNA virus belonging to the *Orbivirus* genus in the *Reoviridae* family which can infect both domestic and wild ruminants. However, little information is available on BTV infection in wild animals. Most of the studies were on North American and European wildlife and only few dealt with African species. In this study blood and serum samples from 182 springboks (*Antidorcas marsupialis*) and 50 blue wildebeests (*Connochaetes taurinus*) were collected and tested for BTV antibodies and RNA by using competitive ELISA and real-time RT-PCR, respectively. To determine the BTV serotype, ELISA positive samples were tested by serum neutralization assay. Bluetongue virus RNA was found in 120 (66%) springboks and 9 (18%) wildebeests. In none of them it was possible to isolate the virus. BTV antibodies were detected in all the wildebeests and in the majority (n=170; 93%) of the springboks tested. Apart from BTV-25 which does not grow in tissue culture and for which it is not possible to perform the SN, all the other recognized BTV serotypes were detected by SN. Interestingly, even though many animals presented antibodies against different serotypes, BTV-1, BTV-16 and BTV-26 were the most frequently found. This survey demonstrated that BTV has widely circulated or is circulating in the Etosha National Park. It was the first time that BTV-17, BTV-20 and BTV-21 were detected in the sub-Saharan Africa. It was also the first time that the presence of BTV-26 was demonstrated outside Kuwait, the country where it was detected. Further studies are needed to elucidate the impact of wildlife on the epidemiology of BTV in the Southern part of Africa with particular emphasis on the newly discovered BTV-26.

Introduction

Bluetongue virus (BTV) is a double strand RNA virus belonging to the *Orbivirus* genus in the *Reoviridae* family. Up to date twenty six serotypes have been recognized, with the latter two, BTV-25 and BTV-26 just recently isolated in goats and sheep in Switzerland and Kuwait, respectively (Hofmann *et al*., 2008; Maan *et al*., 2011). Bluetongue has been historically designated as an African disease and, although it has not been reported from all African countries, it is probably enzootic in most areas of the continent. However, no information on the BTV serotype circulating in Namibia is available and the only existing data regard the South Africa livestock, where 21 BTV serotypes have been reported, with serotypes 17, 20, 21, 25 and 26 never detected. All ruminants are susceptible to BTV, but severity of clinical signs and lesions may vary from species to species. Wild ruminants are susceptible to BTV infection. Some species could also show severe clinical signs following infection as reported in many studies in North American and European deer. No much is known on BTV in African wildlife and on the role that these species may play in BTV epidemiology.

The present study investigates on the presence of BTV in the Etosha National Park providing critical information upon the circulating serotypes.

Knowing on BT in both springboks and wildebeests is important because of the abundance of these species in the mixed farming system that is broadly diffuse in Namibia and in other Southern African Development Community countries (SADC).

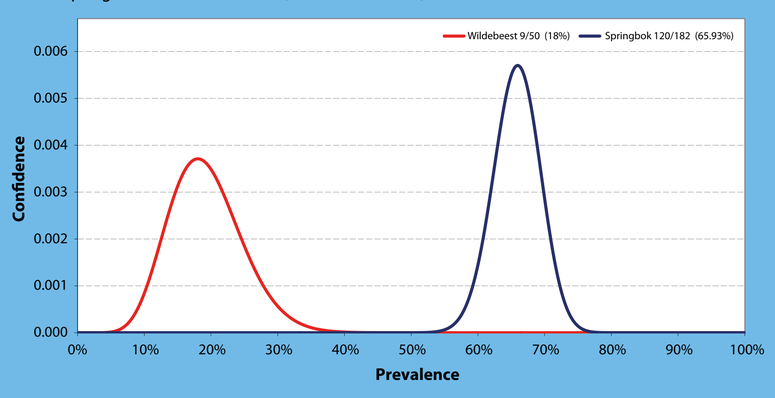
Materials and Methods

Within the surveillance program implemented by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' (IZSAM), the Namibian Ministry of Environment and Tourism (MET) and the Namibian Ministry of Agriculture, Water & Forestry (MAWF) to monitor the circulation of foot and mouth disease virus (FMDV) in springboks (*Antidorcas marsupialis*) and wildebeests (*Connochaetes taurinus*) living in the Etosha National Park in Namibia, 50 wildebeests and 182 springboks were caught and sampled. Animals were randomly immobilized at water holes with a combination of etorphine hydrochloride (M99), azaperone and hyaluronidase. For serological tests, 40 ml of blood were collected in vacutainer serum tubes from the jugular vein while for virology 20 ml of blood were collected in EDTA tubes.
All samples were sent to the laboratories of the IZSAM in Italy to be tested for BTV. The c-ELISA kit manufactured by IZSAM (Lelli *et al*., 2003) was used to detect BTV antibodies in the serum samples whereas the real time RT-PCR as described by Hoffman *et al*. (2008) was used to detect the presence of BTV RNA in EDTA blood samples. Due to the unknown performance of the ELISA test on wildlife sera, all sera were also tested by serum neutralization assay. This gave the opportunity to evaluate the presence of antibodies against 25 out of 26 known serotypes of BTV. It was not possible to test serum samples for BTV-25 antibodies as it is not possible to grow this serotype *in vitro* (Gard and Kirkland, 1993). Virus isolation was also attempted on RT-PCR positive samples according to Savini *et al.* (2004).

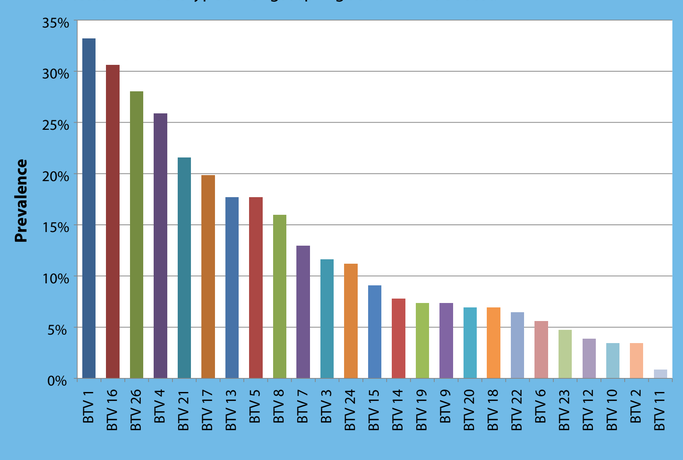
Results

BTV RNA was detected in 120/182 springboks (66%; 95% confidence interval [CI]: 58.77 to 72.43) and in 9/50 (18%; 95% CI: 9.82 to 30.87) wildebeests (**Graph 1**). The attempts to isolate the virus failed. All the sampled wildebeests 50/50 (100%; 95% CI: 94.3 to 100) and 171/182 springboks (94%; 95% [CI]: 89.50 to 96.57) showed BTV antibodies when tested by ELISA. Neutralising antibodies were also detected in all the wildebeests 50/50 (100%; 95% [CI: 94.3 to 100) and in 133/182 springboks (73%; [CI: 66.19 to 78.99). Antibodies against all the tested BTV serotypes were detected at least once in both species (**Graph 2**). BTV-1, BTV-4 and BTV-26 neutralising antibodies were those most often (above 15%) detected in springboks (**Graph 3**), whereas BTV-16, BTV-26, BTV-17, BTV-21 and BTV-5 were those found with higher prevalence (above 40%) in wildebeests (**Graph 3**). One hundred and eighty three animals had antibodies against multiple serotypes (**Graph 4**). Differences were found between the springbok (**Graph 5**) and wildebeest prevalence (**Graph 6**).

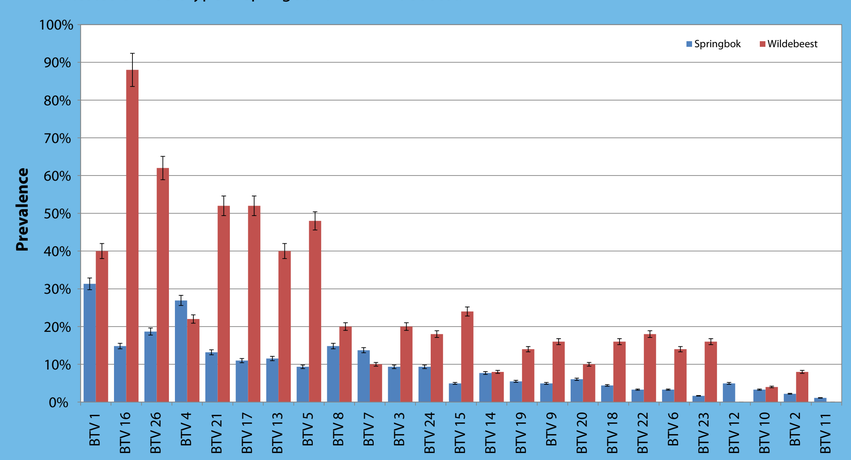
Graph 1. Comparison of Beta distribution of virus prevalence detected by Real Time RT-PCR in springbok and in wildebeest (120/182 and 9/50).



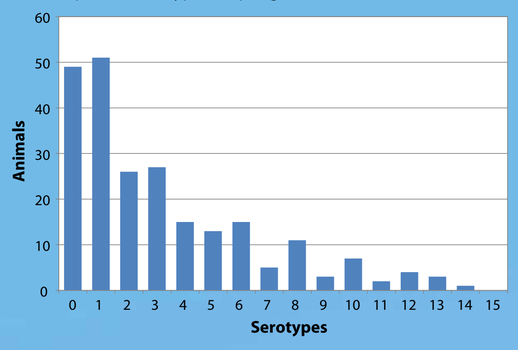
Graph 2. Antibody prevalence, by serum neutralization, against each of the 25 tested BTV serotype amongst springbok and wildebeest.



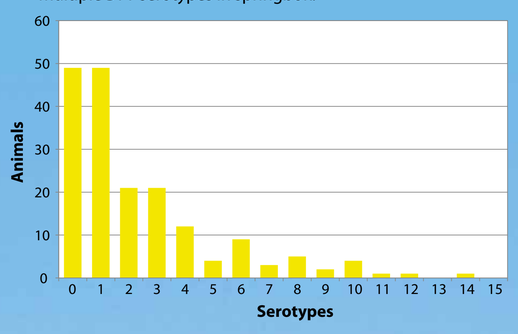
Graph 3. Comparison of antibody prevalence, detected by serum neutralization, against each of the 25 tested BTV serotype in springbok and in wildebeest.



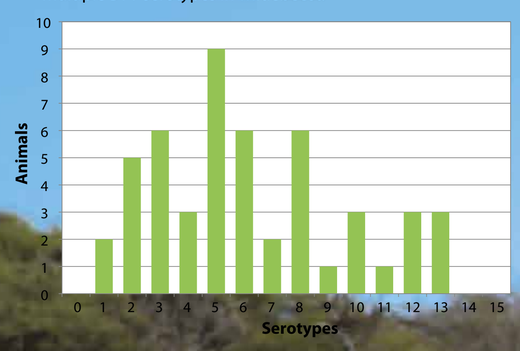
Graph 4. Antibodies detected by serum neutralization against multiple BTV serotypes in springbok and in wildebeest.



Graph 5. Antibodies detected by serum neutralization against multiple BTV serotypes in springbok.



Graph 6. Antibodies detected by serum neutralization against multiple BTV serotypes in wildebeest.



Discussion and Conclusions

The present work first provides information on the BTV serotypes circulating in the Etosha National Park (Namibia). Most of the tested wildebeests and springboks showed either BTV antibodies or RNA. If on one hand this result confirmed the susceptibility of these species to BTV infection, on the other side it revealed the presence of an endemic situation for Bluetongue in which wild ruminants and midges are capable of maintaining the infection in the park environment. This endemic cycle appears to be efficient for all tested serotypes including those never detected in the Southern part of Africa like BTV-17, BTV-20, BTV-21, BTV-26. No data concerning BTV serotype circulation is available for Namibia and therefore, results from this study are the only available. It is the first time that BTV-26 is proved to circulate outside Kuwait and whether it originated in the Sub-Saharan Africa and then spread to Kuwait, or it originated in Kuwait and subsequently spread to Africa, is hard to say. Similarly it is difficult to assess the origin of the other serotype first detected in this part of Africa.

Wild ruminants are naturally subjected to a rapid turnover and within ENP the life expectancy of springboks is generally much shorter than that of wildebeests. This probably explained the significantly lower number of serologically positive springboks compared to the wildebeests and also provides information on possible recent and past infections.

Currently, we are not aware whether, in Namibia, BTV in wildlife follows the pattern observed in domestic animals. This study, however, is a first attempt to identify the factors that might influence the BT dynamics in the natural contest of the sixth largest National Park in Africa.

Further studies, based on a larger number of wildlife species including also domestic livestock, are required to better understand BT epidemiology and to investigate how environmental factors influence vector dynamics at the wildlife/livestock interface.

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