Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (Syncerus caffer) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa

Introduction

Rift Valley fever (RVF) and lumpy skin disease (LSD) are both economically important diseases, initially endemic to sub-Saharan Africa but which have expanded into North Africa and recently the Middle East (Israel, Saudi Arabia, Yemen and Oman) (Abraham & Zissman 1991; Fagbo 2002; Imam, Darwish & El-Karamany 1979). Both diseases have the potential for global emergence (Britch & Linthicum 2007; Tuppurainen & Oura 2012).

Rift Valley fever is a mosquito-borne viral disease of domestic and wild ruminants characterised by necrotic hepatitis and haemorrhages, with often inapparent or mild infections in wild animals. The disease is caused by the Rift Valley fever virus (RVFV), a negative-sense, segmented single-stranded RNA virus of the genus Phlebovirus in the family Bunyaviridae (Rice et al. 1980). Transmission is mainly by Aedes spp. mosquitoes and transovarial transmission via aedine eggs occurs (Linthicum et al. 1985). Many other mosquito genera, such as Anopheles, Culex and Mansonia transmit RVFV (Swanepeol & Coetzter 2004). Outbreaks of RVF in sub-Saharan Africa are usually associated with the emergence of large numbers of aedine mosquitoes following abnormally heavy rainfall with flooding. After virus amplification in vertebrates, mosquito species, such as Culex spp., act as secondary vectors to sustain the epidemic (Linthicum et al. 1985). In northern and western Africa, outbreaks have occurred independently of rainfall and transmission is mediated by river or dam breeding mosquitoes (Swanepeol & Coetzter 2004). The isolation of RVFV from mosquitoes and detection of RVFV-specific immunoglobulin G (IgG) in animal and human populations during inter-epidemic periods are indicative of RVFV activity (LaBeaud et al. 2008; Linthicum et al. 1985; Rostal et al. 2010).

The disease was first reported in 1931 in Kenya (Daubney, Hudson & Garnham 1931) and severe RVF outbreaks, serological evidence and/or virus isolation have since been recorded across sub-Saharan Africa (Olaleye et al. 1996; Ringot et al. 2004; Swanepeol & Coetzter 2004; Zeller et al. 1995). The disease has expanded geographically into Egypt (Imam et al. 1979), Madagascar (Morvan et al. 1992) and Saudi Arabia and Yemen on the Arabian Peninsula (Fagbo 2002). In

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Rift Valley fever and lumpy skin disease are transboundary viral diseases endemic in Africa and some parts of the Middle East, but with increasing potential for global emergence. Wild ruminants, such as the African buffalo (Syncerus caffer), are thought to play a role in the epidemiology of these diseases. This study sought to expand the understanding of the role of buffalo in the maintenance of Rift Valley fever virus (RVFV) and lumpy skin disease virus (LSDV) by determining seroprevalence to these viruses during an inter-epidemic period. Buffaloes from the Kruger National Park (n = 138) and Hluhluwe-iMfolozi Park (n = 110) in South Africa were sampled and tested for immunoglobulin G (IgG) and neutralising antibodies against LSDV and RVFV using an indirect enzyme-linked immunosorbent assay (I-ELISA) and the serum neutralisation test (SNT). The I-ELISA for LSDV and RVFV detected IgG antibodies in 70 of 248 (28.2%) and 15 of 248 (6.1%) buffaloes, respectively. Using the SNT, LSDV and RVFV neutralising antibodies were found in 5 of 66 (7.6%) and 12 of 57 (21.1%), respectively, of samples tested. The RVFV I-ELISA and SNT results correlated well with previously reported results. Of the 12 SNT RVFV-positive sera, three (25.0%) had very high SNT titres of 1:640. Neutralising antibody titres of more than 1:80 were found in 80.0% of the positive sera tested. The LSDV SNT results did not correlate with results obtained by the I-ELISA and neutralising antibody titres detected were low, with the highest (1:20) recorded in only two buffaloes, whilst 11 buffaloes (4.4%) had evidence of co-infection with both viruses. Results obtained in this study complement other reports suggesting a role for buffaloes in the epidemiology of these diseases during inter-epidemic periods.


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in several buffaloes from a LSDV-endemic area in Kenya (Davies 1982). African buffaloes may thus play a role in the epidemiology of LSD.

The LSDV survives for more than 30 days in skin lesions (Tuppurainen, Venter & Coetzer 2005; Weiss 1968) and infection is characterised by fever, multiple firm circumscribed skin nodules and necrotic plaques in the mucous membranes, mastitis, orchitis and swelling of the peripheral lymph nodes (Coetzer 2004). The disease was first reported in Zambia in the late-1930s (Weiss 1968) and reached South Africa by 1944 (Thomas, Robinson & Alexander 1945) affecting some eight million cattle (Backström 1945). It then spread throughout sub-Saharan Africa, characterised by periodic outbreaks (Davies 1991). Outside sub-Saharan Africa, LSD was first documented in Egypt (House et al. 1990), followed by Israel (Abraham & Zissman 1991), with additional reports of serologically confirmed capripoxvirus infection in Saudi Arabia and Oman (capripoxvirus, not confirmed LSDV) (Greth et al. 1992; Kumar 2011). In South Africa, epidemics have persisted and more recent ones in 1989–1990 and 2000–2001 have been reported (Coetzer 2004). Outbreaks also occurred in 2010 in the Eastern Cape, Mpumalanga, Limpopo, Free State, Gauteng, Western Cape and North-West Provinces of South Africa (World Organisation for Animal Health 2010).

Saliva, infected skin lesions and milk have been implicated in the transmission of LSDV (Weiss 1968) and semen has experimentally been shown to transmit LSDV (Annandale et al. 2013). Direct transmission between animals is thought to be inefficient and mechanical transmission by blood-feeding arthropods has been suggested (Chihoita et al. 2001). Recently, transmission of the virus by Ixodid ticks was also demonstrated (Lubinga et al. 2013; Tuppurainen et al. 2013).

Presumptive diagnosis is generally based on clinical signs, but various techniques are available for LSDV diagnosis (Awad et al. 2010; Binepal, Ongadi & Chepkwony 2001; Tuppurainen et al. 2005). The ELISA is most suited for screening large numbers of samples for evidence of past infection. Although the serum neutralisation test (SNT) is time consuming, lacks sensitivity and cannot discriminate between antibodies to the different capripoxviruses, it is a reliable serological test (Babiuk et al. 2009).

Various ELISA protocols are available for LSDV diagnosis in cattle (Carn 1995; Heine et al. 1999; Paweska, Mortimer, Leman & Swanepoel 2005). A recently validated ELISA that detects antibodies to LSDV in cattle using an inactivated sheeppox virus has been used. It is easier to perform, less time consuming and does not require live virus and BSL-3 facilities in LSD-free countries (Babiuk et al. 2009). However, a validated ELISA available for use in wildlife sera is lacking.

This study investigated the seroprevalence of LSDV and RVFV in stored sera of buffaloes obtained from the Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HlIp), South Africa during an inter-epidemic period using an indirect ELISA (I-ELISA) and SNT.
Research method and design

Sample collection

Serum samples were collected between 2003 and 2004 from African buffaloes during a routine examination of animals in the KNP and HiP. Samples were collected from three areas in the KNP: Lower Sabie (twice sampled, once in 2003 and 2004), Gudzani Dam and Satara, as well as from the HiP in the Kwazulu-Natal Province (Figure 1). These samples (Table 1) had been stored since 2003 at -20 °C at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria and were tested for antibodies to LSDV and RVFV.

The KNP is the largest wildlife reserve (approximately 20 000 km²) in South Africa. It has a length and breadth of about 320 km and 65 km respectively. It is bordered by Zimbabwe in the north and Mozambique in the east. The Lower Sabie area consists of the knobthorn savanna and Lebombo bushveld ecozones, whilst the surrounding vegetation at Satara is knobthorn and marula savanna on basalt soils; no specific vegetation description of the Gudzani Dam area could be found (South African National Parks [SANParks] n.d.; Venter & Gertenbach 1986). All three areas in the KNP experience a similar average annual rainfall of between 400 mm and 500 mm, mainly during summer (December, January and February) (SA Explorer 2011; Venter & Gertenbach 1986). As publicised on SANParks’ website for the KNP (http://www.sanparks.co.za/parks/kruger/), the Park supports more than 147 mammalian species, including about 2500 African buffaloes.

The HiP is the third largest (about 900 km²) game reserve in South Africa. It is covered mainly by savanna grassland and the buffalo population (roughly about 3000) forms stable herds (Dora 2004).

Laboratory tests

Lumpy skin disease virus IgG indirect ELISA

The I-ELISA used was carried out as described previously with minor modifications (Babiuk et al. 2009). The cut-off value for the I-ELISA was a standard deviation (s.d.) of +3 of the mean negative control. Each buffalo serum sample was tested twice to achieve quality control. For each plate, there were three replicates of the positive and negative controls and four replicates of the conjugate controls. The internal quality control (IQC) parameters upper control limits and lower control limits were derived from the mean ± 2 s.d. for replicates of each control (Paweska et al. 2003). Furthermore, the coefficient of variation of the positive control on each plate was monitored not to exceed 20%.

Rift Valley fever virus IgG indirect ELISA

This I-ELISA protocol has been described previously and the kits were kindly provided by the Animal Research Council – Onderstepoort Veterinary Institute (Jansen van Vuren et al. 2007). The mean optical density values were calculated into percentage positivity (PP) values for interpretation. Samples with PP values > 10 were regarded as positive. Lower PP values were interpreted to mean that fewer antibodies were detected in the samples.

The IQC procedure previously described was used with minor adjustments (Paweska, Jansen van Vuren et al. 2008). Each buffalo serum was tested twice and each plate had four replicates of each high-positive serum, negative serum and conjugate controls.

Serum virus neutralisation test

Selected positive and negative ELISA sera were tested by the SNT. The SNT procedures for both viruses were

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**TABLE 1**: Information on the buffalo sera used in this study.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of samples</th>
<th>GPS coordinates</th>
<th>Month and year sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gudzani Dam</td>
<td>25</td>
<td>24°37’ S; 31°93’ E</td>
<td>August 2003</td>
</tr>
<tr>
<td>Satara</td>
<td>21</td>
<td>24°39’ S; 31°78’ E</td>
<td>April 2003</td>
</tr>
<tr>
<td>Hluhluwe-iMfolozi</td>
<td>110</td>
<td>28°28’ S; 30°86’ E</td>
<td>September 2004</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>248</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
carried out following the standard operating procedures of the Department of Veterinary Tropical Diseases, Faculty Veterinary Science, University of Pretoria (Beard et al. 2010).

**Lumpy skin disease virus**
The procedure was carried out using 96-well, flat-bottomed cell culture microtitre plates according to the method described by Beard et al. (2010). Bovine dermis cells were used as culturing host and a working virus concentration of 100TCID₅₀ was used. Plates were incubated at 37 °C in an atmosphere containing 5% CO₂ for 14 days. The end point titre was determined from the last dilution where the virus or serum mixture inhibited cytopathic effect (CPE).

To ensure quality control of the SNT’s of both LSDV and RVFV, virus controls were carried out in duplicate on the same plate as some, but not all, of the samples.

**Rift Valley fever virus**
The same procedure for RVFV was used with as with the LSDV SNT, but with minor modifications. Vero cells were used as culturing host. The plates were incubated at 37 °C in a humid atmosphere of 5% CO₂ and observed daily for 4 days. The end point titre was determined from the last dilution where the virus or serum mixture inhibited CPE.

**Ethical considerations**
Samples were collected under supervision of a veterinarian during routine sampling of animals in the KNP and HiP. No animals were culled in the sampling process. All samples were transported to the BSL3 facility of the Agriculture Research Council’s Transboundary Animal Diseases Section where they were inactivated. Serum samples were then brought to the Department of Veterinary Tropical Diseases at the University of Pretoria for analysis in a BSL2 laboratory.

**Results**

**Indirect ELISA**
A total of 248 serum samples were each tested using the two different I-ELISAs. LSDV antibodies were detected in 28.2% (70 of 248) of samples. The highest prevalence was recorded in the HiP where 35.5% (39 of 110) were positive. From a total of 138 samples taken in the KNP, Lower Sabie (2004) had the highest percentage of LSDV I-ELISA IgG (10 of 41; 24.4%). Of the 138 sera from the KNP tested by I-ELISA, 35 (25.4%) were tested for neutralising antibodies to LSDV and 38 (27.5%) to RVFV by the SNT. Only five sera were positive for these antibodies to LSDV, three taken from Lower Sabie in 2003 and two from Gudzani Dam; nine sera were positive for antibodies to RVFV, the majority (88.9%); 8 of 9) of which were from Lower Sabie. None of the sera from Satara tested positive for LSDV (n = 7) or RVFV (n = 4) neutralising antibodies (Table 3). Of the samples from HiP that were tested for both viruses using the I-ELISA, 31 of 110 (28.2%) were tested for LSDV and 19 of 110 (17.3%) for RVFV using the SNT. None of these had neutralising antibodies to LSDV and three were positive for antibodies to RVFV (Table 3).

**Discussion**
Evidence of natural and experimental infections with RVFV and LSDV in wildlife has been documented (Davies & Karstad 1981; Evans et al. 2008; Hedger & Hamblin 1983; Young, Basson & Weiss 1970). In this study, sera were collected from buffaloes during an inter-epidemic period in the KNP and HiP regions and the prevalence of antibodies to both LSDV and RVFV was obtained using I-ELISA protocols. Selected positive and negative sera from the ELISA were then tested using the SNT.

Based on RVFV IgG detection, a prevalence of 6.5% (9 of 138) and 4.5% (5 of 110) in the KNP and HiP, respectively, were obtained in this study. Other studies similar to this one include those of Anderson and Rowe (1998), Barnard (1997), LaBeaud et al. (2011) and Wollhuter et al. (2009). In the earliest study, all 71 buffaloes (from the KNP) tested were negative. Another study from the KNP reported a seroprevalence of 57.6% (117 of 203), although the ELISA used was not described (Wollhuter et al. 2009). The most recent study

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**TABLE 2:** Indirect ELISA results for lumpy skin disease virus and Rift Valley fever virus.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total number of samples</th>
<th>LSDV</th>
<th>RVFV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive samples</td>
<td>Positive samples in %</td>
</tr>
<tr>
<td>Lower Sabie (2003)</td>
<td>51</td>
<td>13</td>
<td>25.5</td>
</tr>
<tr>
<td>Lower Sabie (2004)</td>
<td>41</td>
<td>10</td>
<td>24.4</td>
</tr>
<tr>
<td>Gudzani Dam</td>
<td>25</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>Satara</td>
<td>21</td>
<td>5</td>
<td>23.8</td>
</tr>
<tr>
<td>Hluhluwe-Imfolozi Park</td>
<td>110</td>
<td>39</td>
<td>35.5</td>
</tr>
<tr>
<td>Total</td>
<td>248</td>
<td>70</td>
<td>28.2</td>
</tr>
</tbody>
</table>

LSDV, lumpy skin disease virus; RVFV, Rift Valley fever virus.
TABLE 3: Serum virus neutralisation test results for lumpy skin disease virus and Rift Valley fever virus.

<table>
<thead>
<tr>
<th>Location</th>
<th>LSDV</th>
<th></th>
<th>RVFV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of samples</td>
<td>Positive samples</td>
<td>Positive samples in %</td>
<td>Total number of samples</td>
</tr>
<tr>
<td>Lower Sabie (2003)</td>
<td>14</td>
<td>3</td>
<td>21.4</td>
<td>16</td>
</tr>
<tr>
<td>Lower Sabie (2004)</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
<td>11</td>
</tr>
<tr>
<td>Gudzani Dam</td>
<td>4</td>
<td>2</td>
<td>50.0</td>
<td>7</td>
</tr>
<tr>
<td>Sataara</td>
<td>7</td>
<td>0</td>
<td>0.0</td>
<td>4</td>
</tr>
<tr>
<td>Hluhluwe-iMfolozi Park</td>
<td>31</td>
<td>0</td>
<td>0.0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>66</td>
<td>5</td>
<td>7.6</td>
</tr>
</tbody>
</table>

LSDV, lumpy skin disease virus; RVFV, Rift Valley fever virus; ELISA, enzyme-linked immunosorbent assay.

†, Nine samples not tested due to insufficient sera – all were ELISA positive.
‡, Three samples not tested due to insufficient sera – one ELISA positive and two ELISA negatives.

reported 21.0% prevalence in 550 buffaloes tested from the KNP using only the haemagglutination inhibition (HAI) (LaBeaud et al. 2011). In the study performed in Zimbabwe, where both the IgG ELISA and a HAI protocol were used, 34 of 514 (6.3%) buffaloes were positive (Anderson & Rowe 1998). Other studies using buffalo sera either did not specify when samples were taken (epidemic or inter-epidemic period) or samples were from both periods (Evans et al. 2008; Paweska et al. 2007; Paweska, Jansen van Vuren, Kemp et al. 2008; Paweska, Mortimer, Leman & Swanepoel 2005).

In the present study, 21.1% (12 of 57) of samples tested RVFV-positive by the SNT. Results obtained by previous studies, where larger sample sizes were used, reported by Paweska et al. (2003) (5.8%; 54 of 928); Paweska, Jansen van Vuren, Kemp et al. (2008); and Evans et al. (2008) (7.5%; 77 of 1023) and Evans et al. (2008) (15.6%; 37 of 237), were lower than those obtained in this study. Results obtained by the study of Paweska, Mortimer, Leman and Swanepoel (2005) (20.5%; 53 of 258) are similar to those of the present study. However, that study included samples obtained from an outbreak during 1997–1998 in East Africa, the proportion of which was not stated in the paper.

A high correlation between results obtained by the I-ELISA and the SNT (Pearson’s correlation coefficient) was evident in the studies of Evans et al. (2008) ($R^2 = 0.860$) and Paweska, Jansen van Vuren, Kemp et al. (2008) ($R^2 = 0.882$ Spearman test). A comparable high correlation was also obtained in this study between the SNT titres and the PP values from the I-ELISA. The Pearson’s correlation coefficient was $R^2 = 0.750$. This study provides additional evidence that the rNP-based I-ELISA is a valuable diagnostic tool for RVFV seroprevalence studies in the African buffalo.

From a total of 248 buffalo sera tested, 70 (28.2%) were positive for antibodies to LSDV (ELISA). In previous similar LSDV prevalence studies, African buffalo sera were collected between 1963 and 1996 (Barnard 1997; Davies 1982; Hamblin et al. 1990; Hedger & Hamblin 1983) and the SNT was mainly used to detect neutralising antibodies to LSDV (Barnard 1997); another combined the SNT with the indirect fluorescent antibody test (IFAT) (Davies 1982). In the present study, the LSDV SNT detected neutralising antibodies in 5 of 66 (7.6%) samples, some of them with low titres of, for example, 1:20. In the study by Barnard (1997), 15 buffalo samples from the KNP were tested and although antibodies to LSDV were detected in wildebeest, eland, springbok and impala, no antibodies to LSDV could be detected in buffaloes. The small number of buffaloes tested was probably not representative of the potentially infected buffaloes in the KNP. Davies (1982) detected neutralising antibodies in buffalo sera collected during epidemic and inter-epidemic periods in Kenya, Tanzania and Uganda. He indicated that a subset of the IFAT positive samples (150 of 254; 59.1%) were positive by SNT without giving the exact number. Another study, with a much larger and more diverse sample size than the present study, tested more wild ruminant species ($n = 8$) from different game areas in Tanzania (Hamblin et al. 1990). Although all the 370 buffalo sera tested were negative for LSDV neutralising antibodies, information on disease activity at the time of sampling was lacking (Hamblin et al. 1990). The largest LSDV prevalence study in buffalo tested samples collected between 1963 and 1982 from 11 sub-Saharan African countries and all the samples (1413) were negative for LSDV neutralising antibodies (Hedger & Hamblin 1983).

The SNT is not very sensitive in detecting LSDV neutralising antibodies because of the predominantly cell-mediated immune response to LSDV infection (Babiuk et al. 2009). Additionally, LSDV does not easily grow in cell cultures, which makes the SNT difficult to perform. However, the use of bovine dermis cells, a primary cell culture, in this study did not compare well with results obtained by the I-ELISA. Difficulties encountered with development and evaluation of serodiagnostic tests for capripoxviruses have been in obtaining sufficiently large numbers of well-characterised.

The study performed in Zimbabwe, where both the IgG ELISA and a HAI protocol were used, 34 of 514 (6.3%) buffaloes were positive (Anderson & Rowe 1998). Other studies using buffalo sera either did not specify when samples were taken (epidemic or inter-epidemic period) or samples were from both periods (Evans et al. 2008; Paweska et al. 2007; Paweska, Jansen van Vuren, Kemp et al. 2008; Paweska, Mortimer, Leman & Swanepoel 2005).
sera from different host species (e.g. sheep, goats, cattle and buffalo) to facilitate validation (Timothy Bowden pers. comm., 03 June 2011). There are also a large number of host (including breed, age, sex, previous infection or vaccination history, quality of sera etc.) and laboratory shearppox virus factors that might affect the performance characteristics (diagnostic sensitivity and diagnostic specificity) of any antibody-detecting ELISA. Determining the true exposure status of naturally infected animals is therefore often difficult. It is therefore not unusual to obtain a large percentage of seropositive animals, as in this study, using the ELISA.

The high percentage of LSDV-positive antibody results obtained in this study is, however, a concern. Results are in contrast with other published results, as well as results obtained with the SNT for antibodies against LSDV. Samples were obtained from the field and the possibility that the results by the I-ELISA are false positives cannot be excluded. A validated LSDV-specific ELISA, although difficult to establish, should be used for testing buffalo and other wildlife sera.

Conclusion
This study provides data indicating previous infection by LSDV and RVFV in an African buffalo population in the KNP and HiP during an inter-epidemic period. The role of buffaloes in the epidemiology of these diseases is, however, still not clear. From the results obtained, both the SNT and ELISA tests used for RVFV are sensitive and provide reproducible results. However, further studies are required to evaluate the performance characteristics (sensitivity and especially the specificity) of the I-ELISA assay for the detection of antibodies against LSDV in African buffalo serum in order to detect the true prevalence of LSDV antibodies in buffalo. In particular, a large number of known LSD-negative serum samples should be tested using the I-ELISA.

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Competition of interests
The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors’ contributions
E.H.V. (University of Pretoria) was the project leader and responsible for the project design and writing of the manuscript. J.A.W.C. (University of Pretoria) assisted in the project design and writing of the manuscript. S.F. (Ministry of Health) did the experimental work and assisted in writing the manuscript.

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