Preliminary report of transfrontier disease surveillance in free-ranging buffalo in the Caprivi Strip, Namibia

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Summary
A capture operation to ascertain the health status of free-ranging buffalo (Syncerus caffer) from six areas in the Caprivi Strip in the north-east corner of Namibia is described. In-depth reports on buffalo capture operations and their cost, with detailed descriptions of diseases for research purposes, sampling techniques, field processing of samples and laboratory-related costs are still lacking in the literature. This paper describes materials, methods and the related costs of a disease surveillance operation conducted among buffalo in Namibia. The survey attempted to provide information designed to improve the control of infectious diseases in the Caprivi Strip, a key area bordering Angola, Zambia, Botswana and Zimbabwe.

Keywords
Buffalo, Caprivi Strip, Capture, Namibia, Syncerus caffer, TAD, Transboundary animal disease, Transfrontier disease surveillance.

Introduction
The area studied was the Caprivi Strip and the surrounding Kavango-Zambezi (KAZA) transfrontier conservation area that also spans parts of Botswana, Angola, Zimbabwe and Zambia. The landscape is a patchwork of different land uses, tenure types and boundaries, including country borders, veterinary fences, national parks, communal lands, government land and forest reserves. A variety of ethnic groups and nationals of five countries live in the area, making transboundary management of natural resources a key issue.

Game normally roam through the Caprivi and, every year, a number of wild animals undergo both local and transcontinental translocation for their release into new habitats for conservation or sporting purposes (4, 16). The health status of these animals or the health status of the populations from which they originated is seldom investigated. In 2007, ten buffalo (Syncerus caffer) from four regions of the Caprivi were collared with satellite radio collars and their movement monitored. At the same time, 50 buffalo were immobilised to collect sera for preliminary disease profiling (9). A second phase of this project was planned for October 2009.

Game capture operations are expensive activities. Most of the species usually captured are the largest and most valuable, such as buffalo and other antelope. Disease screening of these animals before movements does not always occur; it depends on the area of origin and on the health regulations of the exporting/importing country (1).

Game capture does not always require darting and immobilisation of the animals; this is one of the biggest limiting factors for collecting biological samples.

Transboundary animal diseases (TADs) are defined by the Food and Agriculture Organization (FAO) as those diseases that are...
of economic and/or food security significance, that, by spreading to other countries, may give rise to epidemics. Their management and control require cooperation among countries (5, 7).

The risk factors for disease transmission between wildlife and livestock relate to the type of ecosystem or eco-zone involved; very acid savannas and high Afromontane eco-zones carry a lower risk compared to tropical savannah where wildlife mixes more readily. Since links between wildlife and livestock in some African areas are seamless, knowledge of management issues and disease ecology understanding is imperative (19).

It was decided to work with buffalo because of their relevance as reservoirs of foot and mouth disease (FMD) virus. These animals move long distances during the annual cycle and, in the area covered by the survey, they can cross four international borders (23). In the recent past, since 2001, several outbreaks of FMD have occurred in cattle in the Caprivi Strip and buffalo have been incriminated for failures in immunisation campaigns, on the assumption of being carriers of FMD serotype variants not present in the vaccine.

In addition, samples were also taken to determine the presence of other infectious agents.

In this preliminary report, immobilisation from the helicopter of a significant number of buffalo, depending on the herds size, from six different areas in Caprivi is described; previous screening for FMD virus was conducted in 1998. In addition to probang for FMD, additional samples were collected and were tested for Brucella, infectious bovine rhinotracheitis and contagious bovine pleuropneumonia, as well as external and internal parasites. The selected diseases occur in Namibian cattle since 1994 (World Animal Health Information Database [WAHID]). African buffalo may be infested with exceptionally large numbers and species of Ixodid ticks (12, 28) and on account of their large size, a high percentage of these ticks are usually adults (11, 13). Buffalo may indeed play a role in the transmission of important tick-borne diseases of livestock, but data on ticks from buffalo and livestock in this key area are lacking.

Materials and methods

The area

Caprivi is a strip of land running east-west in the north-east corner of Namibia, with a depth varying between 30 km and 100 km (Fig. 1). The area falls within the Zambezi Baikiaea woodlands eco-region (6), which is characterised by a mosaic of woodlands, savannahs, grasslands, some of which are seasonally flooded, and forests. The altitude above sea level varies between 930 m and 1100 m and rainfall averages approximately 650 mm per
year, mostly falling between November and April. The average daily temperature is approximately 23°C. In 1996, the human population in the area totalled approximately 115 000 (18) in communal lands and settlements, border parks and other protected areas. Wildlife, livestock and people therefore live in close proximity with one another and conflicts between the various species are common (20). In addition, there is an increased risk of disease transmission at the wildlife/livestock interface (Fig. 2).

The Caprivi Strip is at the centre of the recently proposed Kavango-Zambezi Transfrontier Conservation area (KAZA) which will be one the world’s largest conservation areas, measuring almost 280 000 km²; it will include protected areas and communal lands in Zambia, Zimbabwe, Botswana and Angola, as well as Namibia (www.kazapark.com/).

Control fences, along national borders and within the individual countries, play a critical role in limiting disease spread into domestic stock but their presence constitutes major and serious limiting factors to seasonal natural movements of wildlife (22).

Buffalo were captured and sampled in the areas listed below:
- Eastern Flood Plains, Namibian side of Chobe River
- Bwabwata National Park, Buffalo section
- Bwabwata National Park, Mahango Strip
- Bwabwata National Park, Susuwe North and South sections
- Mamili National Park
- Mudumu National Park.

**Personnel**

Four veterinarians, with different skills and levels of expertise, were involved in the project. Two members were from the Ministry of Environment and Tourism and were responsible for anaesthesia, one was a FMD consultant expert from the United Kingdom and the fourth, from the **Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise ‘G. Caporale’**, was responsible for the collection, identification, recording and storage of samples in the field.

Three veterinary technicians from the Namibian Central Veterinary Laboratory provided support for the capture team and assistance to veterinarians in accelerating procedures.

Three members of the Game Capture Team of the Ministry of Environment and Tourism and representatives of different government and non-governmental organisations participated in the capture operations over a period of 10 days.

Three different teams composed of one veterinarian, one technician and one assistant.
were created. This organisation profile enabled us to accelerate procedures, allowing the teams to work with up to five buffalo at any single time.

**Means of transport**

The entire capture operation was performed with a five-seater Bell Jet Ranger 206 (Fig. 3). A Cessna 182 was also available to provide support to capture operations and to reduce helicopter costs. Wherever possible, the Cessna was used to locate buffalo herds and to find previous collared animals that needed to be immobilised again. Four 4-wheel drive vehicles were also available to transport people to the capture site to save ‘helicopter time’.

**Laboratory equipment and sampling collection**

The need to stay in the field for approximately two weeks meant that a mobile field laboratory needed to be organised which could be set up with ease and moved from place to place in a short period of time. Equipment, consisting of centrifuges, pipettes, tanks and a dry shipper for liquid nitrogen, disposable material, camping tables and chairs and two generators, were moved using a small trailer.

A critical issue was to calculate in advance the amount of liquid nitrogen and proper tanks required to last long enough under field conditions with temperatures of up to 40°C.

To facilitate the collection of samples, sampling kits for each animal were prepared in advance (Fig. 4). All the kit content was labelled in advance and then data transferred onto registration sheets while on the ground.

**Anaesthesia**

All buffalo were darted from the helicopter, using a cartridge rifle loaded with Pneu-Dart darts (Fig. 5). They were loaded using a combination of 8 mg etorphine (M99), 60 mg azaperone (stressnil) and 2 000 IU hyaluronidase (hylase), administered intramuscularly. As an antidote, 24 mg of diprenorphine (M5050) and 50 mg of naltrexone were used (17, 21). Most buffalo were darted using this dosage which was calculated for the young target animals.
identified for the survey. In a few cases, a second dart was necessary and, for less than ten animals, the dose of M99 had to be increased to 10 mg.

Results

The animals were approached by air and/or ground crew as soon as the situation was considered to be safe while the spotter plane circled overhead to monitor the position of the rest of the herd.

A total of 95 buffalo were immobilised, among which 54% were males and 46% females; based on dentition (26, 27), 48% of them were between 3 and 8 years old, while the remaining were equally distributed below and above this age (Table I). All buffalo were darted from the helicopter. A total of 81% were down in 2-15 min with an average time of less than 5 min. Sample collection was usually completed within 20 min. Approximately 83% of the animals were back on their feet in less than 40 min from the darting. Only one animal that was probably already sick, died.

Once under the effect of the anaesthetic, each buffalo was immediately moved into sternal recumbency and blindfolded, while two people held the head to reduce the risk of regurgitation of rumen contents. From each buffalo, probang samples, 50 ml of blood in the absence of anticoagulant and 15 ml of blood in ethylenediaminetetraacetic acid (EDTA) from the jugular vein, a tissue sample from the ear and faeces from the rectum, as well as ticks that could be seen on the animal were collected (Fig. 6). On account of the sternal recumbency position, the largest number of ticks was collected from the perineal area. No tick was observed on the neck and near the ears, and a few animals were inspected in the inguinal region for capture needs.

Ticks identified with a stereo microscope at the Central Veterinary Laboratory in Windhoek, through the electronic key produced by the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD3), are summarised in Table II.

Capture operations never lasted later than noon because of the high environmental temperature. All samples were temporarily stored in a cool box on the helicopter, probang samples were first transferred into the transport media and upon return to the camp, they were immediately split into 1.8 ml criotubes, washed on the outside with citric acid before being frozen in liquid nitrogen. Sera were readily separated and aliquoted into 1.8 ml criotubes and frozen at −20°C. The freezer was connected to a generator or car battery. Blood in EDTA was used to make two blood slides that were fixed in methanol, the remaining blood was then stored in a fridge between 0°C and 4°C.

Faeces were promptly refrigerated at 4°C while ticks were kept at room temperature in 70% alcohol.

The total amount spent for the project was approximately €90 000. The greatest expenses were the transport costs, including helicopter time, and FMD analysis that together represented over two-thirds of the total budget.

Table I

<table>
<thead>
<tr>
<th>Capture site</th>
<th>Male</th>
<th>Female</th>
<th>1 to 3 years</th>
<th>3 to 8 years</th>
<th>&gt;8 years</th>
<th>No. of buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bwabwata National Park, Mahango</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Bwabwata National Park, Buffalo</td>
<td>10</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Bwabwata National Park, Susuwe South</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Bwabwata National Park, Susuwe North</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Eastern Flood Plains</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Mudumu National Park</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Mamili National Park</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>13</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>44</td>
<td>23</td>
<td>46</td>
<td>26</td>
<td>95</td>
</tr>
</tbody>
</table>
Discussion

Preparatory meetings held in Windhoek at the Central Veterinary Laboratory and then every night for the next morning’s activity, enabled participants to become acquainted with the capture areas, critical activities, working needs and types of terrain. Most people had worked with buffalo before and all had been trained to work with animals; this was the key to the success of the operation.

Table II
Ticks collected from buffalo: identification and count

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Sex</th>
<th>Number</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyalomma rufipes</td>
<td>Male</td>
<td>126</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Amblyomma variegatum</td>
<td>Male</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Rhipicephalus evertsi evertsi</td>
<td>Male</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hyalomma truncatum</td>
<td>Male</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>233</td>
<td></td>
</tr>
</tbody>
</table>

The three different teams operating on the ground accelerated procedures to reach buffalo that were not close to each other. Helicopter time was also saved by darting up to five buffalo in the same area and at the same time and not having to wait for an animal to go down. It must be emphasised that most of the capture sites were not accessible by 4-wheel drive vehicles.

Since the drug dosage was approximate and was not based on the exact body weight, the procedure adopted to immobilise the animals was considered successful. The loss of a single animal, which was probably already sick, was consistent with the anaesthesia risk associated with this capture method.

A specifically designed capture sheet ensured proper data collection on the following:
- capture areas
- sex
- age
- collected samples
- anaesthesia information.

A summary data sheet to record all information on the samples had been produced in the field laboratory and then, when in Windhoek, was transferred into electronic format.

Of the 100 buffalo originally targeted, only 95 were captured with an average of 16 animals per area at a cost of about €920 per head.

Since vectors of anaplasmosis, bovine babesiosis, bovine dermatophilosis, bovine ehrlichiosis and heartwater were identified, we underlined the need for further studies on epidemiology and on the socio-economic impact of tick-borne diseases in buffalo on small-scale dairy farms in these areas. In spite of the importance of East Coast fever (ECF) or Corridor disease (CD) and other tick-borne diseases, together with the increasing popularity of keeping dairy cattle, very little is known regarding the epidemiology and socio-economic impact of these diseases in small-scale dairy units (14). The absence of *Rhipicephalus appendiculatus*, the vector of ECF and CD, might be explained by the fact that this tick is strictly seasonal and adults are most
abundant during the rainy season (December to April). The epidemiological significance of tick-borne diseases in carrier buffalo needs to be clarified, particularly in relation to their role in establishing and maintaining new diseases in this key area.

Having disease profiles of buffalo in the Caprivi region will add to the current database of disease in the area and will assist the Ministry of Environment and Tourism and Ministry of Agriculture to identify and implement relevant and effective control measures to prevent the occurrence and spread of disease, particularly at the wildlife/domestic interface (15). It could also enable the Ministry of Environment and Tourism to develop procedures in conjunction with the Directorate of Veterinary Services to allow the movement of live wildlife within the region and, under certain conditions, to the rest of Namibia in the FMD-free area where the market exists for these species (22). Conditions under which the possible export and import of wildlife into and out of neighbouring countries also need to be elucidated.

Namibia is an example of a country that is dependent on the meat export trade. To export animal-derived products, primarily beef, Namibia is obliged to comply with strict animal health regulations (25). The ‘compartmentalisation-with-vaccination’ strategy has enabled trade for many years although it has been interrupted on occasion by outbreaks of FMD in cattle (2, 3, 10).

With the establishment of the transfrontier conservation areas, new policies and important economic evaluations will be needed to guarantee adequate conservation strategies for natural resources while ensuring livestock health and production in the future (8). The dilemma to be addressed now is how wildlife can be conserved as it is the basis of a profitable tourist industry, while simultaneously preserving livelihoods based on livestock production and important economic interests based on meat exports (24).

Due to a recent outbreak of FMD (August 2008) bordering the Mahango Game Reserve, all samples are currently being tested for SAT-1, SAT-2 and SAT-3. Knowledge of which serotypes are present may assist in the understanding of the epidemiology of the disease in the region. Genomic typing of these isolates is likely to throw significant light on the source of the recent FMD outbreak. Data relative to laboratory tests will be the object of a separate report as soon as the information becomes available. Our survey was an attempt to provide information designed to improve the control of infectious diseases that buffalo may transmit to domestic stock, with special reference to FMD.

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- Ministry of Environment and Tourism, Namibia
- Central Veterinary Laboratory, Windhoek, Namibia.

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