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# Antibodies to some pathogenic agents in free-living wild species in Tanzania

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## SUMMARY

A total of 535 sera from eight species of wildlife were collected from different game areas in Tanzania between 1987 and 1989. These sera were tested for antibodies against foot-and-mouth disease, bovine herpes virus types 1 and 2, lumpy skin disease, bovine viral diarrhoea, Akabane, bovine ephemeral fever, bluetongue, enzootic bovine leucosis, African horse sickness and African swine fever viruses and *Brucella abortus* based on the expected species susceptibility. Sera from buffalo *Syncerus caffer*, wildebeest *Connochaetes taurinus* and topi *Damaliscus korrigum* contained antibodies against the majority of the pathogens tested. Antibodies to fewer pathogens were detected in sera from the other species. No antibodies to lumpy skin disease virus were detected in any of the sera examined. African horse sickness antibodies were detected in sera from Zebra and African swine fever antibodies were detected in wart hog. The occurrence of antibodies to these agents suggests that wild species act as reservoirs of infection for some of these pathogens. However, until the susceptibility of individual species is proven by isolation of the aetiological agents their role must remain speculative.

## INTRODUCTION

Tanzania supports large numbers of free-living wild animals and their possible role in the maintenance of infectious agents must be considered in so far as these agents may effect the population dynamics of the species and because such species may be implicated in the spread of disease to domesticated animals.

A total of 535 free-living wild animals comprising eight different species was sampled between 1987 and 1989 as part of European Economic Community/Pan African Rinderpest Campaign (EEC/PARC)-funded wildlife projects and other wildlife research programmes at the Serengeti Wildlife Institute (SWI) in Tanzania. Samples were collected from animals in the Serengeti National Park, the Ngorongoro Conservation Area and the Manyara and Tarangire National Parks in the north, Katavi National Park in the west, Ruaha and Mikumi National Parks in central Tanzania and the Selous Game Reserve in the south-east.

These wildlife sera were examined to determine the prevalence of antibodies to the four endemic serotypes of foot-and-mouth disease (FMD) virus, bovine herpes virus types 1 and 2 (BHV 1 and 2), lumpy skin disease (LSD) virus, bovine viral diarrhoea (BVD) virus, Akabane virus, bovine ephemeral fever (BEF) virus, bluetongue (BT) virus, enzootic bovine leukosis (EBL) virus, African horse sickness (AHS) virus, African swine fever (ASF) virus and *Brucella abortus*.

There have been a few previous studies which have included the measurement of antibodies or the isolation of some of these pathogenic agents from wild species in Tanzania. Antibodies against BHV 1 were demonstrated in sera from buffalo *Syncerus caffer*, impala *Aepyceros melampus*, Thomson's gazelle *Gazella thomsoni*, warthog *Phacochoerus aethiopicus* and wildebeest *Connochaetes taurinus* [1, 2]. An Allerton-like strain of BHV 2 was isolated from a sick buffalo during a severe outbreak of disease in Serengeti National Park and antibodies against this virus were detected in sera from 11 of 13 buffalo sampled from these herds [3]. Antibodies against BHV 2 have also been detected in sera from oryx *Oryx gazella* in Tanzania [4]. In 1987, evidence of antibodies against Akabane virus was recorded in sera from waterbuck *Kobus defassa* and *K. ellipsiprymnus* from Tanzania [5]. Plowright [6] reported the presence of antibodies against BVD in sera from buffalo and eland *Taurotragus oryx* in northern Tanzania. More recently, antibodies against BVD virus have been reported in sera from buffalo, wildebeest and one of three topi *Damaliscus korrigum* in Tanzania (Hyera JMK, Leiss B, Anderson EC, Hirji KN, unpublished observations). The ecology of African swine fever has been extensively studied in East Africa including Tanzania [7, 8]. The purpose of this study was to exploit this collection of wildlife sera fully to obtain information of the current status of some pathogenic agents in Tanzanian wildlife species.

## MATERIALS AND METHODS

### *Collection of samples*

Blood samples were collected from 370 buffalo and 165 other wild species in several different localities throughout Tanzania following chemical immobilization [9]. Sera were decanted and stored at −20 °C.

### *Viruses*

Foot-and-mouth disease virus strains $O_1$ BFS 1860, A KENYA 35/80, SAT 1 BOT 1/68 and SAT 2 ZIM 5/81 were propagated in Roux flask monolayers of baby hamster kidney (BHK) clone 21 tissue culture cells. The BHV 1, infectious bovine rhinotracheitis/infectious pustular vulvo-vaginitis, Colorado strain; BHV 2, bovine herpes mammillitis, TV strain; BVD virus, Oregon C24 strain; and LSD virus, Neethling strain were all propagated in primary or secondary calf kidney cell monolayers. The BT, Akabane and BEF viruses were propagated in monolayer cultures of BHK clone 21 cells. African swine fever virus was propagated in roller tube monolayer cultures of primary pig bone marrow.

### *Serum assay*

Antibodies against the four FMD virus types, Akabane and BEF viruses were measured using a liquid-phase antibody blocking ELISA [10]. A duplicate twofold

dilution series of each test serum was reacted with a constant dose of the respective virus. Unbound antigen was then detected using an antigen capture ELISA. Controls in each test included a positive reference serum of known titre and a virus control from which the 100% absorbance value for the virus was calculated. Serum titres were recorded as the dilution giving 50% of the mean absorbance recorded in the virus control wells. Antibody titres equal to or greater than 1 in 45 against FMD and BEF viruses and equal to or greater than 1 in 22 against Akabane virus were considered positive.

Virus neutralization tests against the BHV 1 and 2 and LSD were made in flat-bottomed tissue culture grade microtitre plates. Secondary calf kidney cells were used to assay antibodies against BHV 1 and LSD viruses [11, 12]. Antibodies against BHV 2 were measured using sheep testis cells [5]. Plates were read microscopically. Virus neutralization titres were expressed as the reciprocal of the final dilution of serum present in the serum/virus mixtures at the 50% end-point [13].

Antibodies against BT virus were detected using competitive ELISA [14]. The BT viral antigen was extracted from infected BHK cells [15] but replacing Triton X with Sarkosyl. The assay involved the competition between a single dilution of test sera and a constant amount of mouse monoclonal antibody for BT antigen passively adsorbed onto ELISA plates. Test sera which inhibited the amount of colour by more than 50% as compared with mouse monoclonal antibody in the absence of test sera were recorded positive.

Antibodies against BVD virus were detected using an indirect ELISA. Viral antigens were extracted from infected cell cultures using a 2% solution of 1-octyl-$\beta$-D-glycopyranoside in phosphate-buffered saline (PBS). Negative control antigens were extracted from uninfected cell cultures using the same method. Alternate rows of the ELISA plates were coated with pre-determined dilutions of BVD positive and negative antigens for 24–48 h in a humidity chamber at 4 °C. Plates were washed and a single dilution of each test serum was added to two positive antigen-coated and two negative antigen-coated wells of the ELISA plate. Plates were incubated for 1 h at 37 °C. After washing, anti-species immunoglobulins conjugated to peroxidase was added to each well and incubated for 1 h at 37 °C. The plates were again washed and colour developed following the addition of a chromogen/substrate solution. The optical density (OD) value for each serum was determined by subtracting the value obtained with the negative antigen from the value obtained with the positive antigen. Values greater than 0·1 were considered positive.

Agar gel immunodiffusion tests using a commercial kit (Leukassay B, Pitman-Moore Inc., Washington Crossing, New Jersey) were used to assay sera for antibodies to EBL virus [16]. Concentrated EBL virus was added to the central well of each gel pattern. Positive reference antiserum was added to alternate wells of the rosette and test serum to the remaining test wells. On one pattern, the test wells of the rosette received the positive reference serum, a weak positive serum and a negative serum. Test sera giving complete lines of identity with the control antiserum were recorded positive.

A competitive ELISA was also used to detect antibodies against AHS virus [17] where dilutions of test sera competed with a constant amount of polyclonal

guinea-pig antisera for AHS antigen passively adsorbed onto the solid phase of ELISA plates. End-point titres were recorded as the dilution of test serum giving 50% of the absorption recorded with guinea-pig serum in the absence of test sera. Serotyping was performed using virus micro-neutralization tests [18] adapted for AHS virus where a constant amount of each virus type was reacted with twofold dilutions of test serum. Virus which had not been neutralized was then detected with BHK cells. Antibody titres were determined using the method of Kärber [13]. By grouping the number of zebra *Equus burchelli* with positive reactions to each AHS serotype in clusters, it is possible to give an objective indication as to the virus type which may be circulating [19].

Antibodies against ASF virus were detected by indirect ELISA [20]. Briefly, ELISA plates passively coated with ASF viral protein 73 soluble antigen were used to screen a single dilution of each warthog serum. Sera recording an OD value greater than twice the mean of a pool of negative pig sera were considered positive.

Sera were screened for antibodies to *B. abortus* antigen at a dilution of 1 in 40 using an indirect ELISA. Lipopolysaccharide *B. abortus* antigen was prepared by phenol/water extraction. Plates were coated with a pre-determined concentration of antigen in PBS for 1 h at 37 °C. After washing a duplicate twofold dilution series of test sera in PBS containing 0.05% Tween-20 and 5% skimmed milk powder (Marvel®, Cadbury) (blocking buffer) was added. Plates were incubated for 1 h at 37 °C. Plates were again washed and rabbit anti-bovine IgG peroxidase conjugate, diluted in blocking buffer, was added and plates incubated for 30 min at 37 °C. Plates were washed and colour developed following the addition of orthophenylene diamine containing 0·05% hydrogen peroxide (30% w/v). The reaction was stopped with 1·25 M sulphuric acid. ELISA titres were recorded as positive when OD values were greater than twice the mean of the control negative serum.

## RESULTS

Sera were collected from 535 free-living wild animals in several widely separated areas throughout Tanzania. The numbers and species sampled in each area between 1987 and 1989 are detailed in Table 1.

Table 2 records the occurrence of antibodies in buffalo sera to each of the viral pathogens tested and *B. abortus*. Antibodies against FMD virus types O, A, SAT 1 and SAT 2 were recorded in buffalo sera from all wildlife areas sampled, although the percentage of sera recorded positive against FMD virus types O (33·3%) and A (12·5%) from Manyara National Park was lower than the other game areas sampled. Buffalo sera from all areas were recorded positive against BHV 1 and 2 and BT viruses, although BHV 1 appeared less prevalent in buffalo from Manyara. There was considerable variation in the number of buffalo sero-positive reactions recorded against Akabane (12·5–74·6%), BEF (0·0–70·0%) and BVD (0·0–71·9%) from different wildlife areas in Tanzania. No antibodies against LSD and EBL viruses were detected in any of the buffalo sera examined. Antibodies against *B. abortus* were detected in all areas except the Mikumi National Park and Selous Game Reserve.

In addition to the buffalo, seven other species of wildlife were examined. Table 3

Table 1. *Number of samples collected from different species in each wildlife area*

| Area | Species | No. of samples and year of collection 1987 | 1988 | 1989 | Total |
|---|---|---|---|---|---|
| 1. Serengeti | | | | | |
| (*a*) North | Buffalo | 32 | 2 | 32 | 66 |
| | Wildebeest | 9 | 24 | 8 | 41 |
| | Topi | 1 | 1 | 1 | 3 |
| | Kongoni | — | 3 | — | 3 |
| | Impala | — | 1 | — | 1 |
| (*b*) Central | Buffalo | 6 | 2 | — | 8 |
| | Wildebeest | 4 | 6 | 2 | 12 |
| | Topi | 1 | 5 | 12 | 18 |
| | Kongoni | 1 | — | 7 | 8 |
| | Warthog | 1 | 5 | 1 | 7 |
| | Waterbuck | — | — | 2 | 2 |
| (*c*) South-west | Buffalo | 14 | — | — | 14 |
| | Wildebeest | — | 19 | — | 19 |
| | Kongoni | 1 | — | — | 1 |
| | Waterbuck | — | — | 2 | 2 |
| | Zebra | — | — | 11 | 11 |
| (*d*) West | Buffalo | 37 | — | 76 | 113 |
| | Wildebeest | 15 | — | — | 15 |
| | Topi | 1 | — | — | 1 |
| | Warthog | — | — | 8 | 8 |
| 2. Tarangire | Buffalo | 19 | — | — | 19 |
| | Impala | 1 | — | — | 1 |
| | Waterbuck | 1 | — | — | 1 |
| 3. Ngorongoro | Buffalo | 20 | — | — | 20 |
| | Wildebeest | 4 | — | — | 4 |
| 4. Manyara | Buffalo | 15 | — | — | 15 |
| 5. Lake Katavi | Buffalo | — | 30 | — | 30 |
| 6. Ruaha | Buffalo | — | 51 | — | 51 |
| 7. Mikumi | Buffalo | — | 15 | — | 15 |
| 8. Selous | Buffalo | — | 19 | — | 19 |

shows the number of each species tested and the number of animals with antibodies against each viral pathogen studied. Anti-FMD virus antibodies were detected in sera from all species except zebra, although the prevalence of each virus type varied for each species. Antibodies against BHV 1 were detected in sera from wildebeest, topi and waterbuck *K. defassa*. Antibodies against BHV 2 and Akabane viruses were detected in sera from all species except impala and zebra and against BEF and BT viruses in sera from all species except warthog and zebra. No antibodies against LSD virus were detected in any of the species examined. A total of 15 sera were obtained from warthog in the Serengeti National Park. Seven of these were from the Seronera area and eight from Masabi. Antibodies against ASF virus were only detected in sera from one unweaned animal at Masabi. Antibodies against the AHS group antigen were detected in 8 of the 11 zebra serum samples. The results from serotyping tests showed that of the 8 positive sera, 4 were positive to AHS type 9, 2 were positive to AHS types 2, 5 and 9 and 1 was positive to AHS types 1, 2, 5, and 9. The remaining sero-positive animal had low antibody titres to AHS types 1, 2 and 6.

Table 2. *The prevalence of antibodies to some pathogens in buffalo from Tanzania*

| Origin | FMDV Serotypes O | A | SAT 1 | SAT 2 | BHV 1 | BHV 2 | LSD | Akabane | BEF | BT | BVD | EBL | *Brucella* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Serengeti | | | | | | | | | | | | | |
| (*a*) North | 50/66* | 53/66 | 61/66 | 65/66 | 61/66 | 65/66 | 0/66 | 17/62 | 15/62 | 58/62 | 23/32 | 0/32 | |
| (*b*) Central | 4/8 | 5/8 | 6/8 | 7/8 | 5/8 | 8/8 | 0/8 | 1/8 | 0/8 | 8/8 | NT | NT | 42/193 |
| (*c*) South West | 8/14 | 10/14 | 13/14 | 12/14 | 14/14 | 11/14 | 0/14 | 5/13 | 12/13 | 1/13 | NT | NT | |
| (*d*) West | 90/113 | 74/113 | 83/113 | 97/113 | 89/113 | 111/113 | 0/113 | 79/106 | 39/106 | 103/107 | 10/75 | 0/75 | |
| 2. Tarangire | 13/19 | 16/19 | 16/19 | 19/19 | 17/19 | 18/19 | 0/19 | 11/19 | 5/19 | 19/19 | NT | NT | 10/19 |
| 3. Ngorongoro | 13/20 | 18/20 | 20/20 | 20/20 | 18/20 | 20/20 | 0/20 | 10/20 | 5/20 | 20/20 | NT | NT | 1/19 |
| 4. Manyara | 5/15 | 2/15 | 14/15 | 10/15 | 7/15 | 15/15 | 0/15 | 7/15 | 8/15 | 15/15 | NT | NT | 2/15 |
| 5. Lake Katavi | 28/30 | 30/30 | 30/30 | 27/30 | 21/30 | 23/30 | 0/30 | 18/30 | 27/30 | 30/30 | 0/27 | 0/27 | 6/30 |
| 6. Ruaha | 46/51 | 51/51 | 48/51 | 49/51 | 34/51 | 47/51 | 0/51 | 28/51 | 27/51 | 41/51 | 0/29 | 0/29 | 2/51 |
| 7. Mikumi | 15/15 | 13/15 | 14/15 | 15/15 | 14/15 | 15/15 | 0/15 | 11/15 | 6/15 | 15/15 | 0/14 | 0·14 | 0/15 |
| 8. Selous | 17/19 | 17/19 | 19/19 | 19/19 | 15/19 | 17/19 | 0/19 | 9/19 | 12/19 | 19/19 | 0/18 | 0/18 | 0/19 |
| Totals | 289/370 | 289/370 | 324/370 | 340/370 | 295/370 | 350/370 | 0/370 | 196/358 | 156/358 | 329/359 | 33/195 | 0/195 | 63/361 |

* Number positive/number tested. NT, not tested.

Table 3. *Prevalence of antibodies to some pathogenic agents in wildlife from Tanzania*

| Species | FMDV Types O | A | SAT 1 | SAT 2 | BHV 1 | BHV 2 | LSD | Akabane | BEF | BT |
|---|---|---|---|---|---|---|---|---|---|---|
| Wildebeeste | 4/98* | 15/98 | 45/98 | 47/98 | 39/98 | 7/98 | 0/98 | 18/98 | 15/98 | 45/98 |
| Kongoni | 3/12 | 1/12 | 3/12 | 5/12 | 0/12 | 2/12 | 0/12 | 4/12 | 4/12 | 10/12 |
| Topi | 2/22 | 3/22 | 6/22 | 8/22 | 5/22 | 2/22 | 0/22 | 4/22 | 6/22 | 3/22 |
| Water buck | 2/5 | 1/5 | 1/5 | 4/5 | 3/5 | 4/5 | 0/5 | 2/5 | 4/5 | 2/5 |
| Warthog | 0/15 | 2/15 | 4/15 | 4/15 | 0/15 | 1/15 | 0/15 | 1/15 | 0/15 | 0/15 |
| Impala | 0/2 | 0/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 1/2 | 1/2 |
| Zebra | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 | 0/11 |

* Number positive/number tested.

## DISCUSSION

This study extends our knowledge of infectious diseases of wildlife in Tanzania and may help in assessing their possible influence on wildlife populations and their potential for causing disease in domesticated livestock.

Previous serological surveys in Southern Africa [21–23] and in Kenya [9] have shown that the buffalo is probably the most important maintenance host for FMD virus. The results obtained in this, the first survey of FMD in Tanzanian wildlife, are similar to the previously referenced studies and suggest that the four endemic serotypes are maintained in buffalo. Although FMD virus types O and A occur widely in cattle in eastern Africa, they have never been associated with virus carrier state in buffalo. Serological evidence of infection of buffalo with types O and A, however, has been found in Kenya [9] and this present survey in Tanzania reveals widespread infection with these types. The presence of antibodies to FMD virus serotypes O and A in sera from 90 to 100% of the buffalo sampled from Katavi National Park is of interest since this area is heavily infested with tsetse fly and therefore virtually devoid of cattle. It has not been established how these buffalo might have become infected with types O and A. Possibly the buffalo range beyond the flood plains of Katavi although no studies have yet been carried out.

The presence of antibodies to BHV 1 and 2 was not unexpected as there is serological evidence that infection is widespread in several species of wildlife in a number of African territories [11, 4]. The occurrence of antibodies to BHV 1 in buffalo, waterbuck, topi and wildebeest is consistent with previous reports in East Africa [1, 2, 24]. These authors also reported antibodies in sera from impala and warthog although this was not recorded in this survey. The sera were also examined against BHV 2 and antibodies were detected in all species except impala and zebra. These results were similar to those recorded earlier with buffalo, wildebeest and waterbuck from East Africa [25]. Although these authors did not detect antibodies in sera from kongoni *Alcelaphus cokei*, topi and warthog, small numbers of these three species have been recorded positive in other African territories [4].

No antibodies to LSD virus were detected in sera of any of the species examined in this survey. These results were in contrast to previous reports [12] where antibodies were recorded in six different species including waterbuck and impala. In addition Davies [26], found antibodies in a high proportion of buffalo samples collected from the high altitude mixed farming areas of Kenya following an outbreak of LSD in cattle in the year previous to his survey. Antibodies were not found in wildlife in this survey in Tanzania where a similar severe epizootic of LSD occurred throughout the northern regions of the country in cattle in the mid 1980s. The negative results recorded in this survey might therefore suggest that if wildlife are involved in the perpetuation and spread of this disease their role is probably transitory.

The absence of antibodies to EBL virus in the 195 buffalo sampled from Tanzania may suggest that either the cattle have not been infected, the buffalo are not readily infected or contact between cattle and buffalo is insufficiently close to allow transmission by biological or mechanical routes. Without further evidence of infection in buffalo it may be considered that this species is not particularly

important in the perpetuation of disease and that if transmission between cattle and buffalo does occur it is a rare event.

Plowright [6] recorded the isolation of BVD virus from a captive giraffe in Kenya and cited the isolation by Provost in 1968 of BVD virus from a sick buffalo shot in the Central African Republic which was involved in an outbreak of disease which affected eland and warthog. These observations suggest that several species of wildlife are involved in the maintenance and spread of BVD. Although in this study only relatively small numbers of buffalo sera were examined, there were considerable differences in the proportion of sero-positive animals from each wildlife area. This observation possibly suggests the virus is only circulating within defined areas in Tanzania.

There was a high prevalence of antibodies against BT virus in buffalo, wildebeest and kongoni which confirms the results reported previously [27] when it was also suggested that these species probably act as maintenance hosts. In addition, we detected antibodies in sera from some topi, waterbuck and impala. The high proportion of antibodies in young buffalo below 2 years suggests the virus is probably still active in wildlife populations.

Antibodies against Akabane virus were detected in sera from most of the wild species examined. This result is similar to that recorded for wildlife in Kenya [28], although in our study antibodies were not detected in sera from the small number of impala and zebra tested. A recent survey of antibodies against Akabane virus in wildlife from a number of different African territories [5] records the presence of antibodies in a further 15 species of Bovidae and one species of Hippopotamidae. Although wildlife are undoubtedly involved in the maintenance of Akabane, no studies have been undertaken to determine the possible significance of this disease on wildlife population dynamics.

In 1975, Davies [29] recorded the presence of neutralizing antibodies to BEF virus in sera from buffalo, waterbuck, wildebeest and kongoni in Kenya with evidence that virus had been circulating in these species between epizootics in cattle. This survey confirms the presence of antibodies in these species and also in topi and impala. Studies in other African territories (Hamblin, C. and Al-Busaidy, S., unpublished observations) have demonstrated the presence of antibodies to BEF virus in several other species, namely eland, tsesebe *Damaliscus lunatus*, lechwe *Kobus leche*, elephant *Loxodonta africana*, sable antelope *Hippotragus niger*, warthog, impala, hippopotamus *Hippopotamus amphibius*, giraffe *Giraffa camelopardalis*, oryx, and Grant's gazelle *Gazella granti*.

The successful transmission of an arthropod-borne virus infection depends on a number of factors which include the density of maintenance host species. The results presented here for BT, BEF and Akabane add to the previously reported data indicating that several species of Bovidae are probably involved in maintenance of these viruses.

As AHS is endemic in tropical and to a lesser extent sub-tropical Africa, it was not surprising that the majority of the zebra from Tanzania were recorded sero-positive. Analysis of these results indicate that AHS serotypes 2, 5 and 9 have been circulating in Tanzania, the most predominant serotype being type 9 which is known to be prevalent in the horn of Africa. Three of the zebra recorded positive reactions against all nine serotypes. This multiple response was probably due to either infection with two or more virus types or cross-relationship between types.

The only bacteriological disease examined in this survey was brucellosis. The results showed this to be present in buffalo throughout most of the game areas of Tanzania. This disease has the potential to influence population dynamics and herd structure by causing abortion and chronic infertility, although at present the effect of this pathogen in wildlife is not known.

The data presented here indicates that the wildlife in Tanzania, like other African countries, is probably involved in the maintenance and persistence of a number of pathogenic agents which also infect domesticated animals. Unless there is a major epizootic which results in severe disease and/or significant deaths, infection with these agents is seldom, if ever, investigated in wildlife. More detailed and extensive studies of wildlife ecology are therefore required to determine the role of these and other agents as pathogens for wildlife and the effect of such agents on their population dynamics.

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