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Serological evidence of camel exposure to Peste des Petits ruminants virus in Tanzania

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Abstract

A repository of camel sera collected in northern Tanzania between June through August 2010 was assayed for antibody against Peste des petits virus ruminants virus (PPRV) known to be widespread in the inter-tropical regions of Africa, as well as in the Arabian Peninsula, the Middle East and Asia. A total of 193 serum samples, collected from 14 herds were tested by competitive enzyme linked immunosorbent assay(c-ELISA) for PPRV antibody detection. The overall individual animal level seroprevalence was low, with mean of 2.6% (5/193) and all positive sera were from homebred camels, with at least one seropositive animal detected in 14.2% (2/14) of herds. Amongst the risk factors/variables examined, camels located in Hai, Kilindi and of age category ≥5-10 years old appeared to be most at risk, with seroprevalences of 15%, 13.3% and 5.1%, respectively. The results indicate that PPR virus is circulating in Tanzania. Despite the low prevalence recorded; however, the potential risk to animal health and economic is of concern; underscoring the need for further research, active surveillance to better understand the epidemiology of the disease in a wider geographical area in Tanzania.

Keywords: Dromedary**,** Serology, Peste Des Petits**,** Risk, Tanzania

Introduction

Disease is an important constraint to increased production of animal food for human consumption in most parts of the world. Diseases limit and in some instances, even preclude the development of viable animal industries wherever they occur. Viral diseases such as Peste des petits ruminants (PPR) are economically important in camel rearing regions (Haroun et al., 2002; Abubakar et al., 2008; Albayrak and Gur, 2010). PPR, a primarily disease of sheep and goats is widespread in the inter-tropical regions of Africa, as well as in the Arabian Peninnsula, Asia and the Middle East, where it has and continues to impact animal health, rural livelihoods and livestock-dependent economic growth (Dhar et al., 2002). Cattle, buffaloes, wild ungulates and pigs are also susceptible to infection but do not exhibit clinical signs and are unable to transmit the disease to other animals (Abraham et al, 2005; Chauhan et al., 2009). However, recent studies in Sudan has shown that camel suffer severely with PPR (Khalafalla et al., 2010). PPRV was also suspected to be involved in the epizootic disease that affected one humped camels in Ethiopia in 1995-1996 (Rogers et al., 2001). Clinical signs of PPR disease in camel are characterized by sudden death of apparently healthy animals and yellowish and later bloody diarrhea and abortion. Other mild signs include sub-mandibular swelling, chest pain and frequent coughing (Haroun et al., 2002; Khalafalla et al., 2010).

PPR is caused by PPR virus (PPRV). The virus is described as a pleomorphic particle (400–500 nm); single non segmented negative strand enveloped RNA virus, 15-19 kilobases in length and helical nucleocapsid and is one of the lymphotropic and epitheliotropic viruses of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, and genus *Morbillivirus* (Gibbs et al., 1979; Murthy et. al., 1995). The genus *Morbillivirus* is differentiated into 4 genetically distinct lineages: lineage 1 and 2 – West Africa, lineage 3 - East Africa and lineage 4 –Middle East and Asia (Shaila et al., 1996; Ozkul et al., 2002; Bailey et al., 2005; Chauhan et al., 2009). Serological studies have shown a geographical wide distribution of PPRV. Seroprevalence in camel vary from 0 - 38% depending on countries or regions investigated (Roger et al., 2001; Abraham et al., 2005; Abubakar et al., 2008). Knowledge on the occurrence and prevalence of PPRV infection in equidae species, including camel, in Tanzania is missing till now. The aim of the present cross-sectional survey was to obtain first data on this subject. This study therefore, reports on evidence of PPRV exposure to camel in Tanzania.

Materials and Methods

This rapid exploratory cross-sectional survey was conducted in camel herds in 8 districts of the Tanga, Arusha, Manyara and Kilimanjaro regions, north Tanzania. These eight districts which covers an area of 52,944 km^2 lies between Latitude 2^o 11' and 6^o 14' South of Equator, and Longitude 35^o 11' and 38^o 26' East of Greenwich and receives an average annual rainfall of 1100 mm which is bimodal in distribution. The long rains fall between March- May and the short rains fall between October–December. The amount and duration of rainfall varies from year to year and from season to season. Temperatures vary between 13^oC to 31^oC through-out the year, the coldest month being July and warmest months being October and March, prior to the rains.

The study subjects were all ages, sexes, indigenous breeds of camel, (one hump camel) reared under extensive husbandry which allows free grazing, usually mixed with livestock from other villages. The list of all camel owners in each district was obtained from District Livestock Office and further validated from the data we obtained from Heifer Project International country office, the main supplier of the camels in Tanzania. Data were collected using semi-structured questionnaire and information asked included herd size, source of animals classified as homebred or brought–in, sex, age retrieved from owner herd record. Other information assessed includes health status at a time of visit classified as healthy or unhealthy. Body condition of camels was assessed visually and rated as poor, fair and good. Field survey and sampling was conducted during the period of June to August 2010 and laboratory analysis of samples was conducted in March 2011.

Approximately 10 ml of blood sample was collected from the jugular vein of each animal in all selected herds using plain vacutainer tube (Becton Dickson, UK). Each sample was labelled using codes describing the specific animal and herd. The tube was set tilted on a table over night at a room temperature to allow clotting. Next morning, the clotted blood in the tubes was centrifuged (at 3000 *g* for 20 min) to obtain clear serum. The obtained serum was stored at -20^oC until their analysis.

A monoclonal antibody (MAb) based competitive enzyme linked immuno-sorbent assay (c-ELISA), obtained from commercial supplier (BDSL, Aryshire, Scotland, UK) was used for the detection of antibodies to PPRV (Anderson et al. 1991). All serum samples tested in the present study were processed in duplicate as per the standard protocol. Briefly, the ELISA plates (Nunc Immuno1-Maxisorb, Cat. A39454) were coated with a 1:3000 dilution of PPRV antigen (50 µl/well) derived from (Madin-Darby Bovine Kidney (MDBK) cell culture and the plates were incubated at 37°C for 1 h with constant agitation. Unbound antigen was washed away using washing buffer (0.01 M, pH 7.4±0.2 plus 0.05% Tween 20) followed with the addition of 45µl of blocking buffer to each of the wells (PBS containing 0.5% PPRV negative control serum and 0.05%Tween 20). Five microlitres (µl) of the test and control serum samples (negative, weak positive and strong positive) were then added (in duplicate) followed with the addition of 50 µl of MAb (except to the conjugate control wells) at a concentration of 1:150 in blocking buffer. The plates were then incubated for 1 h with constant agitation. All of the wells were washed with washing buffer after each incubation period. Rabbit antimouse-horseradish peroxidase (HRPO) conjugate, diluted 1:1000 in blocking buffer was added to each well (50 µl/well) and the plates were incubated for 1 h at 37°C with constant agitation. Substrate solution (*O*-phenylenediamine dihydrochloride containing H_2O_2) was added to each well (50 µl/well) allowing 10 min for a colour reaction to develop. The colour reaction was halted with the addition of an equal volume of 1 M H_2SO_4. The ELISA micro plates were read with an immunoskan reader (Flow laboratories, UK) with an inference filter of 492 nm. The reader was connected to a computer loaded with ELISA Data Information (EDI) software which was used to automate the reading and calculation of the percentage inhibition (PI) values. The OD (Optical Density) values were converted to percentage inhibition using the following formula:

PI = 100 – (OD Control or test serum / OD MAb control) * 100

The samples with PI ≥ 50 % (cut-off) were considered positives for PPRV infection

Statistical analysis

Data collected from each study animal and laboratory analyses were coded into appropriate variables entered and biostatastical analysis performed using Epi-info (version 6.04d, CDC, Atlanta, USA). Categorical data were analyzed by using Chi-square (χ^2) test of independence. In all the analyses, a value of $P<0.05$ was considered significant.

Results and Discussion

Overall 14 herds were visited, sampled and the owner or any household hold member interviewed. The average (mean ± SD) herd size of the investigated units was 24.1±21.9, range, 3-72. The overall herd prevalence based on c-ELISA, was 14.2% (2/14). The prevalence was established in single herd from 2 districts namely Siha and Kilindi. Both herds had between 15-20 camels. The within herd prevalence recorded was ranging from 0% to 15%. One possible explanation for the high prevalence of the disease in

larger herds is that larger herd sizes are often maintained by the introduction replacement stock from outside sources. In disagreement to this observation, seroprevalence of PPR was higher in homebred compared to brought-in camels. It is also possible that the spread of the disease from one herd to another herd from one area to another is almost frequently due to movement of infected animal from an infected herd to a non-infected susceptible herd. Also the nature of camel husbandry system, which allowed camel to intermingle freely with other ruminants at grazing, watering points and market places, the camel population, can serve as a ready source of PPRV infection for the ruminants, especially sheep and goats.

Of the 193 camels examined, 144 (74.6%) and 49 (35.4%) were females and males, respectively. Descriptive statistics of the participating herds and husbandry practices intervention are described in detail by Swai et al. (2011) and the proportions of camel in each category of each variable investigated are detailed in Table 1. Prevalence of serum antibodies to PPRV in camels in this study was found to be 2.6%. This is the first serological evidence for the presence of natural PPR virus infection in camel, extending the spectrum of animal hosts of this viral pathogen in Tanzania (Swai et al., 2009).

A strongly significant association (P<0.001) was observed between the administrative localities where samples were collected and the risk of exposure to PPRV. Higher seroprevalences were recorded in Kilindi (13.3%; 2/15) and Siha (15%; 3/20) and none of the other investigated districts were seropositive. The age of the camels was also strongly associated (P<0.001) with risk of infection that was reflected by the prevalence PPRV antibodies. As a result, higher infection rates were observed in camels of age category ≥5-10 years (5.1%) compared to other age groups. Home bred camels had slightly higher sero prevalence

Table 1: The proportions of camels in each category and mean serum antibody prevalence profiles of each variable investigated during the study (n =193)

Variable	Number examined	%	Prevalence (%)	χ^2	P-value
Administrative districts					
Arumeru	20	10.4	0(0)		
Longido	84	43.5	0(0)		
Monduli	7	3.7	0(0)		
Mwanga	8	4.1	0(0)	23.2	0.0015
Same	12	6.2	0(0)		
Hai	20	10.4	3(15)		
Simanjiro	27	14.0	0(0)		
Kilindi	15	7.5	2(13.3)		
Sex					
Females	144	74.6	4(2.7)		
Males	49	25.4	1(2.0)	0.08	0.799
Source					
Homebred	83	43.0	5(6.02)		
Brought-in	110	57.0	0(0)	6.8	0.031
Healthy status					
Healthy	180	93.3	4(2.2)		
Unhealthy	13	6.7	1(7.6)	1.43	0.231
Body score					
Poor	6	3.1	0(0)		
Fair	102	52.8	4(3.9)		
Good	85	44.0	1(1.17)	1.55	0.460
Age category					
≤1.0 yrs	24	12.4	0(0)		
≥1-5 yrs	51	26.4	0(0)		
≥5-10 yrs	98	50.7	5(5.1)	66.8	0.000
≥10-15yrs	10	5.18	0(0)		
≥15 yrs	10	5.18	0(0)		

of 6.02% (5/83) than brought-in from other sources (P< 0.031). There were no differences in seroprevalences among camel body score status, and across sexes and healthy status (P>0.05).

The seroprevalence rate detected in this survey is comparable to the studies done by Abraham et al., (2005) who reported the seroprevalence in Ethiopia to be 3%, while Ismael et al. (1992), using serum neutralization test in Egypt found the seroprevalence in slaughtered camel stock to be 4.2%. On the contrary, lower seroprevalence (0.3%) in camels in Sudan (Saeed et al., 2010) was recorded. The country-to-country variation in seroprevalence might partly be attributed to differences in management system, health care practices, sample size, study periods, diagnostic test used, climate and presence of or other concurrent disease infection (Bekele, 1999; Khalafalla et al., 2010).

However, the detection of PPRV antibodies in an animal species does not essentially mean a clinical relevance of the infection. Nevertheless, the occurrence of PPRV seropositive animals indicates the virus life cycle exists in Tanzania. It remains to be investigated whether the existence of the virus in camel has any epidemiological significance to virus perpetuation and infection to other animal species in the area under study. Since the present survey was restricted to camels in the northern part of Tanzania, studies on the occurrence and economic impact of equidae PPR are also required in other areas of Tanzania.

In accordance with several authors, the receptivity of the camel to PPR virus appears to be a reality (Abraham et al, 2005; Diallo et al., 2007; Kinne et al., 2010). Arguably, the role of camel as a potential reservoir of PPRV which cause disease of ruminants has not been adequately elaborated (Couacy-Hymann et al., 2002; Diallo et al., 2007). One possible hypothesis that can be advanced from the PPRV antibodies in camel recorded in this survey can be due

-either to an immune humoral response to this virus that expressed only the passage and an immune receptivity of the camel, without any clinical or sub-clinical expressions. Under such situation, probably the detection of these antibodies allows revealing the circulation of the PPRV and other *Morbillivirus* related viruses among the cattle and small ruminants. Thus, in that hypothesis, the disease observed has been caused by other(s) pathogen(s).

-or to susceptibility to a *Morbillivirus* closely related to PPRV, which was non-virulent for the camels. Either virus maintenance in camel could result from an interspecies transfer of PPRV strains from cattle or goats and sheep to camel or through the emerging of a new *Morbillivirus*, serologically related to PPRV. PPR was serologically and Polymerase chain reaction (PCR) confirmed in small ruminants during the 2008 outbreak in Tanzania (Swai et al., 2009).

In conclusion, the findings of this study indicate that PPRV is circulating in Tanzania, confirming natural infection in camel for the first time. Therefore, further investigations are, however, needed on virus isolation, genome characterization and determine if it play any potential infection role to other ruminants.

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