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Peste Des Petits Ruminants (PPR) in the Sudan: Investigation of Recent Outbreaks, Virus Isolation and Cell Culture Spectrum

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Abstract: The occurrence of peste des petits ruminants (PPR), which was reported to cause high losses in sheep and goats in the Sudan, was investigated in four different states (Khartoum, Gezira, White Nile, River Nile and Kordofan). The disease affected sheep only. Cattle, goats and in some cases camels grassing alongside sheep did not show any signs of disease. The morbidity and mortality rates varied from one area to another with a mean of 27.2% and 14.9 %, respectively. Relatively higher mortality rate (21%) was recorded in Northern State where the disease appeared for the first time. PPR virus (PPRV) antigen was detected in samples from all areas using agar gel precipitation test (AGPT). PPRV was isolated in primary lamb kidney cells and identified by AGDT and immunocapture ELISA. To determine cell culture spectrum, calf kidney, lamb testicle, chick embryo fibroblast, BHK and VERO cells were inoculated with reference as well as local isolate of PPR. Rapid growth of the virus was seen in chick embryo fibroblast and BHK cells.

Key words: PPR, sheep, Sudan, epidemiology, virus isolation, cell cultures

Introduction

Peste des petits ruminants (PPR) is a highly contagious and infectious viral disease of domestic and wild small ruminants caused by a morbilli virus of the family Parmyxoviridae. The first outbreaks of the disease in sheep and goats that had occurred in Sudan were reported as rinder pest (RP) in three areas in southern Gadarif (eastern Sudan) in 1971 and in goats in central Sudan during 1971-1972 (El Hag Ali, 1973). Since then outbreaks of PPR continued to be reported; in Darfur, central Sudan and Khartoum States by El Hassan *et al.* (1994) and Zeidan (1994), respectively.

The objectives of this work are to elucidate the current situation of the disease in Sudan especially in areas in which the disease had not been reported before and to compare the severity of the disease in term of morbidity and mortality rates. We also intend to use immunocapture ELISA for the rapid and accurate diagnosis of the disease and to compare the growth characteristics of local isolates of PPR in different types of cell cultures and to select the more susceptible cell type for virus isolation.

Materials and Methods

PPR Viruses and Sera

PRV NIG 75/1 Strain: This is a reference PPRV originally isolated from an outbreak in Nigeria in 1975 (El Hag Ali and Taylor, 1984) and was obtained from the virus collection of the Central Veterinary Research Laboratories (CVRL), Soba, Sudan.

PPR VHL Strain: This is a local PPR isolate recovered from sheep from El Hilalia area (El Hassan *et al.*, 1994), it undergone two passages in lamb kidney, two passages in bovine kidney cells and four passages in VERO cells.

PPR Hyperimmune Serum: Rabbit hyperimmune serum produced by Animal Health laboratory, Pirbright, UK was kindly supplied by Dr Emad Aradaib, Department of Medicine, Faculty of Veterinary Medicine, University of Khartoum..

Field Work: Outbreaks of PPR that occurred in Gezira, White Nile, Khartoum, Kordofan and River Nile states during the period 1999-2001 (Table 1) were investigated. Data on clinical signs, morbidity and mortality rates were collected.

Lymph nodes, spleen, nasal and ocular discharges were the main samples collected to detect the presence of PPRV. The samples were taken aseptically, kept in sterile containers and preserved and transported to the CVRL.

Preparation of Samples: The samples were homogenized in mortar and pestle with the aid of sterile sand and prepared as 20% in phosphate buffered saline (PBS) with antibiotics. Supernate fluids after light centrifugation (2000 X g) were collected in sterile bottles and stored at -20°C.

Table 1: The incidence of PPR outbreaks in Sudan, their location, date of occurrence, and the morbidity and mortality rates

Area	Month, year	Morbidity (%)	Mortality (%)
Azaza (Gezira)	February, 1999	24	11.5
Gitaina (White Nile State)	April, 2000	22	15
Abudelaq1-2 (Khartoum State)	June, 2000	30	17.2
CVL(Khartoum State)	June, 2000	33.3	13.3
Soba (Khartoum State)	July, 2000	23	10
Goaz Hammad (N-Kordofan State)	September, 2000	27	12
Kuku (Khartoum State)	February, 2001	27	19
Eldamar (River Nile State)	March, 2001	31.5	21
Mean (%)		27.2	14.9

Detection of PPR Antigen in Suspected Samples by Agar Gel Precipitating Test (AGPT): The test was conducted according to the procedure described by Anderson *et al.* (2000). Original and tissue culture propagated samples were tested by AGPT with and without the addition of 2% sodium deoxycholate.

Virus Isolation in Cell Culture: Lamb kidney cell culture was prepared in Glasgow minimum essential medium (G-MEM) supplemented with 10% fetal bovine serum. One ml of supernate fluid of sample under test was inoculated into 25-cm^2 disposable plastic cell culture flask seeded with confluent monolayer following standard techniques. CPE was examined microscopically daily and when no CPE was detected after 25 days post inoculation (PI), the cells were harvested by 3 cycles of freezing and thawing and cell laysate was blindly passaged in a new set of cells. Cell culture harvests of samples that showed CPE were examined by AGPT and Immunocapture ELISA, to detect the presence of PPRV.

Identification of Local PPRV Isolates by Immunocapture ELISA: An immunocapture ELISA kit produced by Animal Health laboratory, Pirbright, UK was used. The test was performed according to the fact sheet supplied by the distribution company (BDSL, Scotland, UK).

Growth of PPRV Isolates in Different Cell Culture Cell Types: Primary cultures of calf kidney; chick embryo fibroblast; lamb testicle as well as BHK and VERO cell lines were prepared in G-MEM following standard cell culture techniques and inoculated with local isolate if PPRV to determine the biological infectivity of the virus.

Results and Discussion

Epidemiology: The main clinical signs observed in sheep affected with PPV in the present study were pneumonia, diarrhea, and ulceration of mouth, salivation and lacryimation and emaciation. Only sheep of all ages were affected. Cattle, goats and in some cases camels grassing alongside sheep did not show any signs of disease. The morbidity rate ranged between 22 and 33.3% with a mean of 27.2. The mortality rate ranged between 10-21% with a mean of 14.8% (Table 1). The morbidity and mortality rates varied from one area to another. The highest mortality rate was recorded in ELDamer area of northern Sudan, probably due to the absence of PPR outbreaks for several years that results in increased flock susceptibility. Previous exposure to the disease results in solid immunity, which may last for up to 4 years (Durojaiye, 1984) and maternal immunity in kids may last up to 8 months (Rashwan *et al.*, 1996). Variations in morbidity and mortality rates caused by PPR in Sudan were previously also observed (El Hag Ali,1973 and El Hassan *et al.*, 1994).

In this study we confirmed the occurrence of the disease in other geographical areas in the Sudan that were not previously involved including Kordofan states in the west and River Nile state in the north, demonstrating the widespread nature of the disease. As can be noticed in Table 1 the first disease outbreak seemed to originate south of Gezira state in early 1999 and spread westward to involve Kordofan states and northward to involve Khartoum state and later reached northern Sudan in March 2001. This indicated the important role that animal movements and transport plays in disease dissemination and spread. As mentioned by Murphy *et al.* (1999), animal transport imposes stress on the transported animals and introduces diseases to other animals.

Detection of PPR Antigen in Suspected Samples: In the present PPR outbreaks were confirmed as had been caused by PPRV using AGDT, virus isolation and immunocapture antigen detection ELISA. The first outbreaks of PPR in the Sudan were originally diagnosed as RP in sheep and goats by AGDT using RP rabbit hyperimmune serum (El Hag Ali, 1973). We examined lymph node and spleen specimens from suspected animals by AGPT with positive reaction with two precipitin lines. This agrees with Hamdy *et al* (1976) who reported appearance of two precipitin lines when suspected antigen in crude samples was examined by the AGPT. However, precipitin lines appeared

only with tissue culture propagated isolates when treated by antigen extraction agent, sodium deoxycholate (SDC) (Table 2) in agreement to Madboly, *et al* (1987) in using antigen extraction agents with tissue culture propagated PPRVs in AGDT,

To confirm the isolation of PPRV, we used immunocapture ELISA that clearly differentiate between PPR and RP. In the case of diseases such as RP and PPR, which have the same geographical distribution in Africa, the diagnostic assay should be virus-specific (Libeau *et al.*, 1994). Anderson *et al.* (1996) illustrated the great importance of the test in differential diagnosis of PPR and RP as rinderpest and peste des petits ruminants diseases have similar clinical signs and may affect the same animal species. It is therefore, highly recommended to replace AGPT by immunocapture ELISA for routine diagnosis of PPR in Sudan. Besides its high specificity ELISA is rapid and sensitive.

Growth Suspected PPRV in Lamb Kidney Cell Culture: CPE induced by isolated PPRV were similar to that caused by known PPRV strains (Nig.75/1 and PPR VHL) in lamb kidney. Stellated retractile rounded cells were the first changes observed. These cells then aggregated forming syncytia, which increased in number by time. Syncytia were characterized by their clock-face appearance because of the arrangement of nuclei at its edges and may contain few to a large number of cells. Evacuated and floated rounded cells were also seen. It was noted that the CPE began to appear at the edges of the cell sheet then merged to the center. These CPE were similar to what (Hamdy *et al.*, 1976; Gibbs *et al.*, 1977; Gibbs *et al.*, 1979; Rossiter *et al.*, 1985 and Abu El Zein *et al.*, 1990) had reported.

Table 2: Agar gel precipitation test on original specimens, known and local isolates of when examined with and without addition of 2% SDC and when different dilutions of hyperimmune serum were used

		Number of Precipitin Lines					
		With 2% SDC			Without 2% SDC		
Type of Specimen	No. of Samples	conc. HS	1/2 HS	1/10 HS	Conc. HS	1/2 HS	1/10 HS
Original lymph nodes and spleens	9	2	1	1*	2	1	1
Known cell culture propagated PPRV strains	2	1	1	1*	_	_	_
Local PPRV isolates	9	1	1	1			

Conc. HS: Concentrated hyperimmune serum.
* :Faint and close to hyperimmune serum well.
_ :No precipitin lines appeared.

Table 3: The time of CPE appearance when different cell types were inoculated with known PPR viruses and different filed isolates

	Time of CPE appearance (Days)					
Type of Virus	LKC	LTC	BKC	CEF	VERO	BHK
Nigeria (Reference)	10	17	10	1	10	2
PPR VHL (known)	11	19	10	1	11	2
Azaza (field)	11	18	11	1	12	2
Gitaina (field)	12	19	13	1	14	2
CVL (field)	13	19	12	2	14	2
Kuku (field)	12	19	14	2	13	3
Abudelaq1 (field)	11	18	12	1	13	2
Abudelaq2 (field)	15	20	14	3	15	4
Soba (field)	15	20	15	3	14	4
Goaz Hammad (field)	14	20	13	1	14	3
El Damar (field)	13	19	13	1	14	2

LKC: Lamb kidney cells.
LTC: Lamb testicle cells.
BKC: Bovine kidney cells.
CEF: Chick embryo fibroblast.
VERO: African green monkey kidney cells.
BHK: Baby hamster kidney cells.

Table 4: Summary of time elapsed in days post inoculation before appearance of CPE and when CPE involving more than 80% of cell sheet of different types of cells inoculated with reference strains and local isolates of PPRV

	Days Post Inoculation	
Cell Type	Appearance of CPE	CPE involving more than 80% of cell sheet
Primary Lamb Kidney Cells	10-15	15-25
Primary Bovine Kidney Cells	10-15	15-25
Primary Lamb Testicular Cells	17-20	>25
VERO cells	10-15	15-25
Primary Chick Embryo Fibroblast	1-3	4-7
Baby Hamster Kidney Cells	2-4	6-9

Growth of PPRV Isolates in Different Cell Culture Cell Types: All types of cells that were infected with known and 8 local isolates of PPRV in the present study developed typical CPE of PPRV with variation in severity and time appearance of CPE (Table 3). The CPE appeared within 1-20 days post inoculation depending on the type of cells used (Table 4). According to the type of cells used, the CPE took four to twenty five days to involve more than 80% of cell sheet (Table 4).

There is a general agreement that lamb kidney, goat kidney, bovine kidney and VERO cells are the cells of choice for the isolation of PPRV (El Hag and Taylor, 1984; Losos, 1986; El Sanousi *et al.*, 1989; Abu El Zein *et al.*, 1990 and El Hassan *et al.*, 1994). Lamb kidney, goat kidney and bovine kidney are cells of natural host. In the present study, chick embryo fibroblast (CEF) and Baby hamster kidney (BHK)cells showed faster appearance of CPE that involved more than 80% of cell sheet within 1-4 days and 4-10 days, respectively (Tables 3 and 4). On the other hand, in primary LKC and BKC the CPE appeared ten to fifteen days PI and later appeared 8-12 days post inoculation in the second passage. Accordingly, CEF and BHK cells can be added to the list of the cells of choice for inoculation of PPRV and we recommend them for routine PPRV isolation and propagation because of their superior permissiveness to PPRV. Andrews and Pereira (1972) reported the possibility of RP virus cultivation in primary cultures of cattle, sheep, goat, and chick embryo tissues and continuous lines of hamster, and monkey cells. Kingsbury *et al.* (1978) preferred the cultivation of morbilli viruses in primary cultures than in continuous cell line. Lamb testicular cells showed poor CPE after a long time. Therefore, it is not cells of choice for tissue culture isolation of PPRV.

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