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Serological Survey for Peste Des Petits Ruminants Virus (PPRv) in Camel from Different Regions in The West of Libya

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Abstract: ***Peste des petits ruminants (PPR) is an acute feverish viral, disease of sheep and goat characterized by mucopurulent nasal and ocular discharges, necrotizing and erosive stomatitis, enteritis and pneumonia. The disease causes large economic losses each year due to the high rates of mortality and morbidity in infected sheep and goats. In this study, we investigate the sero incidence of PPR in camel flocks in west labia. Serum samples from 492 camel were collected . sero incidence was resolute using virus neutralizing test (VNT) and direct enzyme linked immunosorbent assay (ELISA) . the results of this study showed that the presence of PPR virus antibodies as of west labia sub region , 112 (22.8%) and 380 (77.2%) serum samples were positive and negative for PPRV antibodies respectively.***

Keywords: Serological survey, Peste des petits ruminants, Camel

1. Introduction

Peste des petits ruminants (PPR) is an severe feverish viral disease of small ruminant characterized by mucopurulent nasal and ocular discharges, necrotizing and erosive stomatitis, enteritis and pneumonia [1] . The disease is endemic in Africa and causes large economic losses each year due to the high rates of mortality and morbidity in infected sheep and goats [2 -3] . Morbidity and mortality risks in small ruminants vary but can be as high as 100 and 90%, respectively. These risks are usually lower in endemic areas and mortality can be as low as 20% maintained in the newborns [4] . So that Peste des petits ruminants has been the most important disease of these species in Africa, the Middle East and southwest Asia , it was first described in West Africa in 1942 [5] and later found in Senegal [6] , Central Africa [7], Sudan [8],East Africa [9], Ethiopia [10] . It also results in subclinical infection in large ruminants, which act as carriers of infection to small ruminants [11 -12]. PPR disease is caused by a virus has been classified under genus *Morbillivirus* in the family *Paramyxoviridae* along with other members including rinderpest virus (RPV), measles virus (MV), canine distemper virus (CDV) and morbilliviruses of marine mammals and is antigenically close to rinderpest [13 - 14] . continuous outbreak of PPR occur since more than 30 years affecting sheep , goats and camels [15 – 16 -17] . Camels were not regarded as possible hosts for PPRV until 1992, when a number of authors reported PPRV sero conversion in these animals [18-19] .The first documented outbreak of PPRV in camels, reported from Ethiopia in 1996, consisted of highly contagious respiratory syndromes with high illness rates but low death rates [20-21] . PPR antigen was detected in an outbreak of respiratory disease in camels [20] . Khalafalla detected a new emerging morbillivirus in camels causing respiratory outbreak in Eastern Sudan, the virus was proved to be Peste des petits ruminants (PPR) [22] . Serological surveys have demonstrated that camels are susceptible to the infection and in some instance may express a serious illness (respiratory distress) and mortality[23] .

Our objective was to determine antibody seroprevalence to PPR of unvaccinated camels and to evaluate the role of camels in transitions of the virus between the small ruminant

2. Materials and Methods

2.1. Tissue culture

African Green Monkey Kidney (VERO) cells [24] were cultured in Minimal Essential Medium Eagle (MEM–E-Gibco Laboratories NY USA) supplement with10% fetal bovine serum (Gibco Lab) were used in this study. .The cells were seeded at density of 3× 10^4 per well into 24 –well micro titer plate.

2.2. Peste des petits ruminants virus

Egyptian strain (egypt-87) according to Samia *et al,* [25] was used for propagation at a multiplicity of infection (MOI) 0.5-0.7 per cell in VERO cells . The culture fluids were harvested 36 hours post-infection, clarified by centrifugation during 20 minutes to 12,000 g at 4ºC and used for preparation of the antigen used in direct ELISA and seroneutralization assay [26] .

2.3. Antigen preparation

PPR virus antigen was prepared as per the protocol described for preparation of rinderpest antigen [27] . Briefly, 48-h-old VERO cell monolayers were infected with PPR virus at a multiplicity of infection of 0.5. The culture was harvested at 80–90% cytopathic effect. The culture was freeze-thawed three times and the cell debris was clarified by centrifugation at 1000×*g* for 15 min. The chilled culture supernatant was subjected to precipitation using 8% (w/v) PEG 6000 in the presence of 2.3% (w/v) sodium chloride. The mixture was centrifuged at 8500×*g* for 30 min following overnight incubation at 4 °C. The pellet was dissolved in TNE buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.4) in 10%

of the original volume. PEG concentration was repeated twice to concentrate the material 100 times. The resulting antigen was lyophilized for later use in the standardization of ELISA.

2.4. Serum Sample

Twenty six farms were randomly chosen for this study . these farms distributed in different cities in the west of labia. The serum samples collected haphazardly from each farm represent 15% of camel residents in each farm the samples consisted of 492 camel, over one year old, not vaccinated by rinder peste vaccine and Peste des petits ruminants vaccine. All serum sample was fractionated and inactivated at 56 ºC through 30 minuts for testing in microserum neutralization (MSN) assay and frozen at -20 ºC until they were used.

2.5. Virus Neutralization Test (VNT)

Neutralizing antibodies to rinderpest virus was detected in a micro-assay as described by Bandyopadhyay *et al* , [28] for detection of antibodies to PPR virus , a similar assay was performed using VERO cells. The serum neutralization titer (SNT) was expressed as the reciprocal of the highest dilution of the antibodies that inhabited 50% of viral cytopathic effects according to the method of Reed and Muench, [29] .

2.6. The direct enzyme linked immunosorbent assay (ELISA)

It was performed according to the method previously described by Anderson *et al,* [30] . The antigen was prepared from the infected Vero cell cultures with PPR virus as previously described and diluted with bicarbonate buffer (9.6) using ten fold serial dilution. From each dilution 100 ul were dispensed \ well horizontally and the plates were covered and incubated at $37C^0$ for one hour (coating). The plates were washed three times with the washing solution and 100 ul of the blocking buffer were added \well. After that, the plates were incubated at $37C^0$ for 30 minutes. Then washed three times again with the washing solution. Two fold dilutions of the tested sera were prepared and 100 ul of each diluted serum were added to each well vertically. Positive control sera were included in each plate. The plates were incubated at $37C^O$ for one hour, then washed two times with the washing buffer and 100 ul of the diluted conjugate (1\10000) were dispensed in each well the plates were incubated at 37C for one hour then washed three times with washing buffer and 100 ul of the substrate were add \ well and the plates were incubated at room temperature in a dark place for 15 minutes. The reaction was stopped by adding 25 ul \well of the stopping solution (1.25M).

3. Results and Discussion

The 492 serum samples were randomly selected from twenty six farms and examined using VNT and the direct enzyme linked immunosorbent assay for the presence of PPR virus antibodies as of west labia sub region , 112 (22.8%) and 380 (77.2%) serum samples were positive and negative for PPRV antibodies respectively (Table 3). These results were similar to those found by Abdul-Dahiru *et al* , [31] .

This result quadrate to the results produced in the FAO project TCP/RAB/3302, the mean serological prevalence rates registered 2013 were 34.5 % (25 % in local animals and 45 % in imported heads from e.g. Sudan, Chad, Mali; 25 % in animals < 12 months and 41 % in animals older than two years). Higher serological prevalence rate was observed in southern provinces, probably due to significant uncontrolled animal movements .

The seroprevalences of PPR virus antibodies varied between sub regions of west labia. the highest seroprevalence recorded in Elgamel city 28.6% and the lowest in Sebrata city 11.8% (Table 1 & 2) .These results were parallel to those found by Abraham *et al,* [32] .

The sub regions of west Labia (El ogylat , Zewara , El gamel , El zawia , Sebrata) examined for the presence of PPR virus antibodies, the results of the VNT revealed PPR antibody prevalence rates of 25.2 % , 15.7 % ,28.6 % ,23.8 % ,11.8 % for sub regions of west Labia respectively. While the direct enzyme linked immunosorbent assay showed a seroprevalence of 26.2 % , 17.6 % ,28.6 %, 23.8 % , 11.8 % for sub regions of west Labia respectively (Table 1 & 2) .
This study is the first study to report the seroprevalence of PPR among the camels populations in the sub regions of west Labia. The existence of circulating PPR antibodies attributed to that the spread of the disease from one herd to another herd and 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 [11 - 12] . so that the camels may have served as a bridge with areas of northern Africa and contributed to the spread of a camel PPR virus .

Table 1: The results show the number of camel sera (n =492) with negative or positive neutralizing antibodies against PPRV in each farm and the titer of positive sample using VNT

Region	Farm number	Seropositives of SNT titer					% of Seropositives of SNT	Seronegatives of SNT	
		2	4	8	16	32			Total number of serum sample
		Number of serum sample							
El ogylat	1	2	2	1	1	0	35.3 %	11	17
	2	4	2	1	1	0	30.8 %	18	26
	3	1	1	0	0	0	22.2%	7	9
	4	2	1	0	0	0	23.1%	10	13
	5	2	2	1	0	0	21.7 %	18	23
	6	2	1	0	0	0	15.8 %	16	19
Total		**13**	**9**	**3**	**2**	0	25.2%	80	107
Zewara	7	1	1	0	0	0	16.7%	10	12
	8	1	0	0	0	0	6.3 %	15	16
	9	1	1	0	0	0	20 %	8	10
	10	1	0	1	1	0	23.1 %	10	13
Total		**4**	**2**	**1**	**1**	0	15.7%	43	51
El gamel	11	3	2	1	2	0	38.1 %	13	21
	12	2	2	3	1	0	27.6 %	21	29
	13	2	0	1	1	0	23.5 %	13	17
	14	4	2	1	0	0	31.8 %	15	22
	15	1	0	0	0	0	11.1 %	8	9
Total		**12**	**7**	**5**	**4**	0	%28.6	70	98
El zawia	16	3	3	2	1	0	39.1%	14	23
	17	4	3	1	1	0	30%	21	30
	18	2	0	0	0	0	11.1%	16	18
	19	3	2	1	0	0	28.6%	15	12
	20	3	1	2	0	0	23.1%	20	26
	21	0	0	0	0	0	0%	10	10
	22	2	2	1	1	0	18.8%	26	23
Total		**17**	**11**	**7**	**3**	0	23.8%	122	160
Sebrata	23	1	2	0	0	0	15 %	17	20
	24	2	1	1	0	0	21.1%	15	19
	25	0	0	0	0	0	0 %	14	14
	26	1	1	0	0	0	8.7 %	21	23
Total		**4**	**4**	**1**	**0**	0	11.8%	67	76

Table 2: The results show the number of camel sera (n =492) with negative or positive neutralizing antibodies against PPRV in each farm and the titer of positive sample using ELISA

Region	Farm number	Seropositives of ELISA titer					% of Seropositives of ELISA	Seronegatives of ELISA	Total number of serum sample
		2	4	8	16	32			
		Number of serum sample							
El ogylat	1	2	2	1	1	0	35.3%	11	17
	2	4	1	1	1	1	30.8 %	18	26
	3	1	0	1	0	0	22.2%	7	9
	4	1	1	1	0	0	23.1%	10	13
	5	2	1	1	1	0	21.7 %	18	23
	6	3	1	0	0	0	21.1 %	15	19
Total		13	6	5	3	1	26.2%	79	107
Zewara	7	1	0	1	0	0	16.7 %	10	12
	8	2	0	0	0	0	12.5 %	14	16
	9	0	2	0	0	0	20 %	8	10
	10	1	0	0	1	1	23.1 %	10	13
Total		4	2	1	1	1	17.6%	42	51
El gamel	11	3	2	1	1	1	38.1 %	13	21
	12	1	3	3	1	0	27.6 %	21	29
	13	1	1	0	2	0	23.5 %	13	17
	14	4	1	2	0	0	31.8 %	15	22
	15	1	0	0	0	0	11.1 %	8	9
Total		10	7	6	4	1	28.6%	70	98
El zawia	16	3	2	3	1	0	39.1%	14	23
	17	3	4	1	1	0	30 %	21	30
	18	1	1	0	0	0	11.1%	16	18
	19	2	1	1	1	1	28.6%	15	12
	20	3	1	1	1	0	26.9%	20	26
	21	0	0	0	0	0	0%	10	10
	22	2	1	2	1	0	18.8 %	26	23
Total		14	10	8	5	1	23.8%	122	160
Sebrata	23	1	1	1	0	0	15 %	17	20
	24	1	2	1	0	0	21.1%	15	19
	25	0	0	0	0	0	0%	14	14
	26	1	0	1	0	0	8.7 %	21	23
Total		3	3	3	0	0	11.8%	67	76

Table 3: The results show the Percentage of each titer , Seropositives and Seronegatives of camel sera (n =492) to neutralizing antibodies against PPRV using ELISA

	Seropositives of ELISA titer					Total number of Seropositives of ELISA	Total number of Seronegatives of ELISA
	2	4	8	16	32		
Total	44	28	23	13	4	112	380
Percentage	8.9%	5.7%	4.7%	2.6%	0.81%	22.8%	77.2%

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