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Prevalence of *Peste des petitis ruminants* Infection in Sheep and Goat Farms at the Central Region of Saudi Arabia*

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Abstract: A survey was carried out to investigate the prevalence of *Peste des petits ruminants morbillivirus* (PPRV) infection in sheep and goats during the period from September 2005 to March 2006, at the central region of Saudi Arabia. Competitive ELISA was employed to detect antibodies in serum samples collected randomly from sheep and goats in 11 governorates in the study region. Peste des petits ruminants infection was evidenced in 363/992 (36.59%) and 530/962 (55.09%) of the tested sheep and goats, respectively. The overall prevalence varied between 16 and 69.5% in the different governorates. Sera collected from camels and cattle grazing the same area of infected sheep and goats were negative to PPRV antibodies and therefore could not have played a role in disease transmission.

Key words: *Peste des petits ruminants*, competitive ELISA

INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious systemic disease of sheep and goats which is caused by a morbillivirus that is closely related to rinderpest of cattle (Diop *et al*., 2005; Roeder *et al*., 1994; Awa *et al*., 2000). The course of the disease might be peracute, acute, or chronic and after an incubation period of 5-6 days, clinical signs develop, including fever, anorexia, necrotic stomatitis, gingivitis and diarrhoea (Lefevre and Diallo, 1990; Anonymous, 2000). *Peste des petits ruminants* virus might lead to high economic losses where mortality in goats could reach 95% and in sheep ouly slightly less (Murphy *et al*., 1999). PPR occurs in most African countries, the Middle East and the Indian subcontinent (Abraham *et al*., 2005; El-Hag and Taylor, 1984; Lefevre, 1982; Lefevre *et al*., 1991; Taylor, 1984). The PPR virus infection as well as its isolation has been reported in Saudi Arabian (Al-Afaleq *et al*., 2004; Abu Elzein *e al*., 1990; Al-Naeem *et al*., 2000; Özkul *et al*., 2002). However, epidemiological data of the disease in the country are lacking.

In the present study, an ELISA serosurvey was conducted on sheep and goats belonging to different flocks in Qassim and neighboring regions of central Saudi Arabia to confirm or disconfirm the clinical suspicion of PPR infection. Serum samples were also obtained from camels and cattle to investigate their role in PPR transmission to sheep and goats in close contact.

MATERIALS AND METHODS

Samples

Serum samples were collected from 992 sheep, 962 goats, 461 camels and 353 cattle belonging to flocks of 11 governorates of Qassim and neighboring regions of the central Saudi Arabia (Table 1). The samples were randomly collected from animals of different ages and breeds during the period from September 2005 to March 2006. Collected samples were frozen at -20°C pending serotesing with ELISA.

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Table 1: Governorates surveyed and number of serum samples collected from different animal species investigated

	No. of collected samples*			
Governorate	Sheep	Goat	Camels	Cattle
Shaqraa	100	100	50	50
Onayzah	100	100	50	50
Buraydah	100	100	50	-
Al-Bikairyah	40	25	50	50
Al-Badaye	52	47	11	6
Oyon Al-Jawa	100	100	50	50
Ryad Al-Khabra	100	100	50	2
Al-Shamasyah	100	100	50	32
Al-Butain	100	90	-	13
Al-Zolfi	100	100	50	50
Murbak	100	100	50	50
Total	992	962	461	353

*: Samples were collected from clinically-diseased and normal animals of different ages

ELISA Kit

A competitive enzyme immunoassay kit, for detection of antibody to PPRV, was obtained from Cirad-emvt (Centre de cooperation internationale en recherché agronomique pour le development, Department d'elevage et de medicine veterinaire, Montepellier, France). The kit has been prepared in collaboration with the Animal Production and Health Section of the Joint FAO/IAEA Division on Nuclear and Related Techniques in Food and Agriculture. The test depends on inhibition of the binding of a mouse monoclonal antibody (mAb), directed against the nucleoprotein (N) of the PPRV, in the presence of positive serum. The contents of the kit are microelisa plates (Nunc immuno I Maxisorp), recombinant N-RPPRV antigen stock (0.5 mL vials), anti-RPPV mAb stock (1 mL vials), rabbit anti-mouse immunoglobulin horseradish peroxidase (HRPO) conjugate (Dako, P 260), Control strong positive, weak positive and negative serum stocks (C++, C+ and C-) supplied in 1 mL vials, orthophenylenediamine (OPD) tablets as a chromogen stock and perhydrit tablets as a stock substrate. Antigen, mAb and control serum stocks were supplied in lyophilized forms to be reconstituted when needed. Coating, blocking and washing buffers as well as stopping solution were prepared according to the kit supplier instructions.

Competitive ELISA

Competitive ELISA was carried out on serum samples following the instructions of the supplier where the method was developed after Libeau *et al*. (1995). N-PPR antigen was diluted in coating buffer (PBS, 0.01 M, pH 7.4) according to the antigen batch number. Each well of a microtiter plate was charged with 50 µL diluted antigen followed by 1 h incubation at 37°C on an orbital shaker. After 3 washings with washing buffer (PBS with 0.05% tween 20) and blot dryness, 45 µL of blocking buffer (PBS + 0.05% tween 20 + 0.5% negative lamb serum) was added to all wells of the plate. According to the manufacturer plate layout, the followings were added: 5 µL of blocking buffer to the monoclonal control wells, 55 µL of blocking buffer to the conjugate control wells and 5 µL of test, strong positive, weak positive and negative control sera were added to the corresponding wells. Fifty microliters of mAb (diluted 1/100 in blocking buffer) was added to all wells except the conjugate control ones followed by incubation of the plate for 1 h at 37°C on orbital shaker. After 3 washings and blot drying of the plate, 50 µL of anti-mouse conjugate (diluted 1/1000 in blocking buffer) were added to all wells followed by 1 h incubation at 37°C on orbital shaker. After 3 washings, 50 µL of the chromogen/substrate mixture (OPD/H_2O_2) were added to all wells. After 10 minutes incubation at room temperature, colour development was stopped by adding 50 µL of stop solution (H_2SO_4, 1 M) to all wells. Optical Density (OD) values were read at 492 um with an ELISA reader (Flow Laboratories, England).

Interpretation

The inhibition of mAb binding in the presence of serum was expressed as Percentage Inhibition (PI), calculated from mean OD values using the formula:

$$PI = 100 - [(OD \text{ of the test wells/OD of mAb control wells } 0) \times 100]$$

Sera with PI greater than 50% were considered to be PPR positive.

RESULTS AND DISCUSSION

The prevalence of PPR in an enzootic area may be similar to that of rinderpest (RP) in that a low rate of infection exists continuously (Anonymous, 2002). When a susceptible population builds up, periodic epizootics (outbreaks) occur, some of which might lead to almost 100% mortality among affected goat and sheep at risk (Taylor, 1984; Lefevre and Diallo, 1990).

The ELISA test was significantly sensitive in the diagnosis of PPRV infection and recommended as a suitable alternative to virus isolation (Saliki *et al*., 1994). In the present study competitive ELISA was employed to test serum samples of sheep and goats for presence of antibodies against PPRV. Serum samples from camel and cattle were tested for antibodies against PPRV based on reports of the possible role of wild animals and cattle in transmission of PPRV (Furley *et al*., 1987). As shown in Table 2, out of 992 serum samples of sheep, 363 were found to be positive for presence of antibodies against PPRV with prevalence of 36.59%. Out of 962 goats 530 were found PPR-positive with prevalence of 55.09%. As there was no history of vaccination is undertaken against PPR infection in sheep and goats in the investigated flocks, these findings indicated active infection of the positive cases.

In Shaqraa and Onayzah governorates, PPR seroprevalence rates in goats were 71 and 68% while in sheep the rates were 68 and 66%, respectively. This indicated that the PPRV infection was prevalent and more endemic in these governorates. On the other hand, the prevalence rates of the disease in the rest of the governorates ranged between 10 and 48% (Table 2).

Results have shown that the prevalence of PPRV in goats was higher than in sheep. Both field and laboratory observations indicate that PPR is less severe in sheep than in goats (Taylor, 1984; Lefevre and Diallo, 1990). The mean prevalence of PPR infection in sheep and goats was lowest in Al-Bakairyah governorate and highest in Shaqraa, governorate (Table 2). This could be due to the better veterinary services offered at the relatively advanced Al-Bakairyah governorate. Poor nutritional status, stress of transport and concurrent parasitic and bacterial infections were incriminated in enhancing the severity of clinical signs of PPR (Taylor, 1984; Lefevre and Diallo, 1990). The recorded prevalence of PPR infection in goats and sheep at central Saudi Arabia are alarming and necessitate more investigation for better control strategy.

Table 2: Prevalence of *Peste des petits ruminants* infection among sheep and goats at the central region of Saudi Arabia

	Percentage of positive cases		
Governorate	Sheep	Goats	Mean (%)
Shaqraa	68.00	71.00	69.50
Onayzah	66.00	63.00	64.50
Buraydah	22.00	48.00	35.00
Al-Bikairyah	20.00	12.00	16.00
Al-Badaye	38.46	53.19	45.82
Oyon Al-Jawa	41.00	42.00	41.50
Riyadh Al-Khabra	38.00	44.00	41.00
Al-Shamasyah	17.00	28.00	22.50
Al-Butain	10.00	30.00	20.00
Al-Zolfi	46.00	41.00	43.50
Murbak	27.00	40.00	33.50
Mean	36.59	55.09	45.84

Camels and cattle grazing with infected sheep and goats were negative for PPR infection and they played no role in the disease transmission.

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