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PESTE DES PETITS RUMINANTS AMONG SHEEP AND GOATS IN SAUDI ARABIA IN 2004

(With 4 Tables)

By

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**طاعون المجترات الصغيرة في الاغنام والماعز
بالمملكة العربية السعودية في ٢٠٠٤ م**

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تم تسجيل اندلاع مرض طاعون المجترات الصغيرة في الإغنام والماعز في المنطقة الوسطي والمنطقة الجنوبية من المملكة في الفترة ما بين أبريل إلى اغسطس لعام ٢٠٠٤م. لوحظ ظهور أعراض المرض الاكلينيكية النمطية من حمى وفقدان شهية واسهال وافرزات انفية عينية مخاطية صديدية وصعوبة التنفس والتهاب الفم القرصي التكرزي بالإضافة وجود معدل نفوق مرتفع نسبياً في الحيوانات الصغيرة أعمار ٤-٩ شهور. وكانت أهم الافات التشريحية في الحيوانات النافقة جراء الإصابة بالمرض هي الالتهابات الرئوية والمعوية ووجود أنزفة وتكزز في الغشاء المخاطي للمعدة الرابعة والالتهاب البلوري. تم استخدام اختباري الفلورسنت المناعي الغير مباشر واليزا الاسر المناعي القياسي في الكشف عن الفيروس في عينات كحاتات أفات الفم وأنسجة الطحال والرئة. وقد أوضحت النتائج جاهزية وسرعة وحساسية الإختبارين. وتم استخدام اختبار الإليزا التنافسي القياسي للكشف عن الأجسام المناعية النوعية للفيروس في أمصال الاغنام والماعز المصابة (التشخيص المصلي) وقد اعطت نتائج ايجابية بنسب تراوحت ما بين ٤٠,٧% إلى ١٠٠%. وقد خلصت الدراسة إلي أن اندلعت مرض طاعون المجترات الصغيرة هي في الغالب نتيجة لوجود عترة ضارية حقلية تسدور بين قطعان الحيوانات المصابة والقابله للإصابة مع عدم وجود نظم تحصين حقيقية ضد المرض. وتوصي الدراسة بتنفيذ تقنين حظر حركة الحيوانات اثناء فترة خطر انتقال العدوه في مناطق المملكة المختلفة ووضع برنامج تحصين وطني إلزامي ضد المرض في الإغنام والماعز على امتداد المملكة وأن يتم تحصين المواليد بعد بلوغها عمر ٣ شهور حين تدني أوتلاشي المناعة الإمية بها.

SUMMARY

An outbreak of Peste des petits ruminants (PPR) among sheep and goats was reported in the central and southern regions of the Kingdom of Saudi Arabia between April-August 2004. Typical clinical manifestations of acute PPR including, fever, anorexia, diarrhea, mucopurulent nasal and ocular discharges, dyspnea and necro-ulcerative stomatitis were observed with a relatively high case fatality rate among lambs and kids aged 4-9 months. On postmortem examinations, pneumonia, enteritis, hemorrhages and necrosis in the abomasal mucosa, and pleurisy were the most common gross lesions. Both PPRV-antigen and antibody assays were used to confirm diagnosis of the disease. Indirect fluorescent antibody (IFA) technique and immuno-capture ELISA (IC-ELISA) were used for demonstration of PPR viral antigen in the mouth scraping, spleen and lung tissue specimens. The laboratory study indicated that both IFA and IC-ELISA were reliable, rapid and sensitive methods for detection and identification of PPRV antigen. Competitive ELISA (C-ELISA) was used for detection of PPRV-antibodies in ovine and caprine sera. Serodiagnosis revealed that 40.7-100% of the collected sheep and goats sera were positive. In conclusions, the reported PPR outbreak is mostly attributed to the presence of enzootic virulent strains of PPR virus, which could have circulate between the infected and susceptible animals in sheep and goats flocks with no actual national vaccination regime against the disease. The present study recommended that, legalization of the restriction of the animal movement should be applied for sheep and goats populations during the risk period of PPR in different localities of the country. An obligatory vaccination program against PPR virus infection should be applied for all sheep and goats flocks all over the country. Lambs and kids should be vaccinated at 3rd month of age, when the maternal antibodies decline.

Key words: *Competitive ELISA (C-ELISA) - Indirect fluorescent antibody (IFA) technique - Immuno-capture ELISA (IC-ELISA) – Outbreak - Peste des petits ruminants (PPR) – PPR virus – Serodiagnosis - Vaccination*

INTRODUCTION

Peste des petits ruminants (PPR) is an acute contagious viral disease of goats and sheep characterized by necrotizing and erosive stomatitis, enteritis and pneumonia. The causative agent, PPRV, is a member of the family Paramyxoviridae and the genus Morbillivirus.

Various strains of PPR virus are of one antigenic type (Gibbs, *et. al.*, 1979). There is existence of a close antigenic relationship between PPR and Rinderpest (RP) viruses (Hamdy, *et. al.*, 1975; Diallo, *et. al.* 1994).

The disease is prevalent in West Africa, the Middle East and south-west Asia (Taylor, 1984; Taylor, *et. al.*, 1990; Shaila, *et. al.*, 1996). In recent years, PPR has emerged in Turkey (Özkul, *et. al.*, 2002).

PPR is enzootic in Saudi Arabia, outbreaks variably occur in susceptible sheep and goats populations particularly in animals over 4 months and under 1 year of age (Abu-Elzein, *et. al.* 1990; Al-Naeem, *et. al.* 2000, Abdel Baky, *et. al.*, 2001). In most countries in which PPR is enzootic, policy of vaccination is considered the most suitable control measure.

There have been several millions of sheep and goats are raising in different localities in Saudi Arabia. Since 2000, the veterinary vaccine production center in Riyadh is unique in annual manufacturing of approximately 4.5-6 million doses of live attenuated vaccine of PPR virus, strain Nigeria 75/1 but almost no actual national park for vaccination against PPR in Saudi Arabia until now has been taken to control the disease in sheep and goats.

The objective of this study is to record the data of epizootiological and virological aspects of PPR during 2004 outbreak and discuss the benefits of organizing national park for vaccination against PPR in different localities in Saudi Arabia for controlling of the disease.

MATERIALS and METHODS

Epizootiological data

Data of the most observed clinical signs, postmortem findings, morbidity and case fatality rates, and history of vaccination against PPR in the affected flocks were recorded in details.

Samples for laboratory diagnosis

Serum samples and tissue specimens from spleen, lung and/or mouth lesions scraping were collected from animals showing clinical signs of PPR pre- and post necropsy. The samples were sent in ice box to the laboratory of veterinary diagnosis in Riyadh where subjected for laboratory investigations for detection of antibodies against PPR virus and for detection of the viral antigen as well as for isolation of the virus.

Preparation of tissue specimens

Tissue specimens were prepared for virus isolation and detection of the viral antigen. An appropriate piece of chosen affected tissue was

cut and washed in cold 0.01 M phosphate buffer saline (PBS), pH 7.4, then minced by sterile scissor to very small pieces and homogenized in PBS to prepare 10-20% tissue homogenate (W/V) using electric homogenizer, Power Gen 125, Fisher Scientific USA. During the homogenization process, the container of the homogenate was kept cold by dipping in ice path. The homogenate was then sieved via four layers of sterile wet gauze and centrifuged at 3000 rpm for 10 minutes at 4 °C.

The supernatant fluid of the centrifuged homogenate was divided into two portions, the first portion was filtrated via 0.45 µm Ø Millipore syringe filter, Flowpore, ICN and kept at -70 °C until used for PPR virus isolation. While the second portion was used directly for detection of PPR viral antigen by immuno-capture enzyme linked immunosorbent assay (IC-ELISA). Sediment of the centrifuged homogenate was washed twice with PBS through resuspension and centrifugation. The washed cells were dispersed and diluted in appropriate volume of PBS and dispensed in wells of chamber slide, Lab-Tek, Nalgen Nunc (50 µl/well). After dryness of the cell suspension on the wells of the chamber slide, 100 µl of cold 80% acetone in PBS was added to each well and kept at -20 °C for 20 minutes for fixation. After washing once with PBS, the fixed cells on the slide become ready for application of indirect immunofluorescent technique for detection of PPR viral antigen.

Cells and virus

Cultures of primary ovine embryo kidney cells and vero cell lines propagated in Eagles minimal essential medium (E-MEM) supplemented with 5-10% of fetal calf serum (ICN Biomedicals, INC, Ohio, USA) plus pencillin sodium and streptomycin sulphate at final concentration of 100 µl and 100 ug/ml media were used for trails for virus isolation from the received tissue specimens.

Attenuated strain of PPRV, Nigerian 75/1 of titer 6.0 log₁₀TCID₅₀/vial kindly supplied by veterinary vaccine production centre, Riyadh, KSA was used as a control virus of known infectivity titer in immuno-capture ELISA.

Laboratory investigations

Indirect fluorescent antibody (IFA) technique

IFA was applied to visualize PPRV antigen in infected tissue specimens in guidness of Woldehiwet and Hussin (1994) as follows:

Fixed cells on the slides were incubated with a 1/5 dilution of normal rabbit serum in 0.01 M PBS, pH 7.4 (50 µl/well) for 10 minutes at 37 °C. After thorough washing in PBS for 10 minutes at room temperature, standard strong anti-PPR sheep serum as well as standard PPR-negative

sheep serum Pribright, UK, diluted 1:20 in PBS was added to the slides (50 μ l/well). The slides were placed in humid box at 37 °C for 1 hour. After washing twice, 50 μ l of 1/100 dilution of fluorescein-conjugated rabbit IgG fraction to sheep IgG, Eappel, Organon, Teknika Crop, West Chester was added to each well and followed by incubation at 37 °C in humid box for 45 minutes. After another thorough twice washing, the slides were examined. Specificity of immunofluorescence was evaluated by first examining of cells exposed to standard negative PPR sheep serum.

Immunocapture ELISA (IC-ELISA)

Commercial Immunocapture ELISA kit for detection of PPRV- and RPV-antigens in supernatant of the centrifuged tissue homogenate has been developed by Pribright laboratories, UK. Test kit was used according to the manufacture's recommendations. The test is based on the technique of Libeau *et. al.* 1994. A mouse monoclonal antibody (anti-PPRV/RPV) that attached to the microplate capture the virus present in tested sample. A second mouse monoclonal antibody (anti-PPRV/RPV) which is biotinylated and is directed against the nucleocapsid (N) protein of the representative virus is used in conjugation with streptavidine-horseradishperoxidase conjugate to detect the capture antigen. Results were expressed as optical density values and related to a cut off value calculated according to manufacture's instruction.

Virus isolation

Trails for isolation of the causative virus from mouth-lesions scraping, spleen and lung tissue specimens throughout one passage in ovine embryo kidney cell cultures and three passage in vero cell cultures following the method of Taylor and Abegunde (1979).

Competitive ELISA

PPR competitive ELISA (C-ELISA) kit was prepared in collaboration between Cirad-emet laboratories and the animal production and health section of the joint FAO/IAEA. It used for detection of specific antibodies against PPRV in the serum samples collected from the affected animals. The test depends on inhibition of the bindings of mouse monoclonal antibodies to a PPRV-specific epitope in the presence of positive serum. Inhibition is detected as a reduction in the optical density reading obtained with the monoclonal antibody alone following the addition of peroxidase labeled anti-mouse conjugate and substrate/chromogen mixture. Tested sera demonstrating mean percent of inhibition (PI) values of $\geq 50\%$ are considered to be positive and

tested sera demonstrating mean PI values less than 50% are considered to be negative. The test is based on the technique of (Libeau, *et. al.* 1995)

RESULTS

Clinical manifestations

The most frequent clinical manifestations of PPR reported in 13 sheep flocks and 11 goat flocks with laboratory confirmed disease in Saudi Arabia between March and August 2004 are represented in table (1). From this table, it is clear the most common recorded clinical signs of PPR in the affected sheep and goats were fever, anorexia, diarrhea, nasal and ocular discharges dyspnea, and necro-ulcerative stomatitis at frequency rates of (100%), (92.3% and 100%), (84.6% and 100%), (84.6% and 90.9%), (61.5% and 81.8%), and (69.2% and 54.5%) respectively.

Necropsy findings

At postmortem (PM) examinations, the most observed gross lesions in sheep and goats were pneumonia, enteritis, and inflammation of the abomasal mucosa in frequency rate of (90.9% and 90%), (81.8% and 90%) and (18.2% and 30%) as shown in table (1).

Table 1: Frequency of PPR clinical signs and gross lesions reported in 13 sheep flocks and 11 goat flocks with laboratory confirmed disease in Saudi Arabia between March and August 2004.

Signs/lesions	Sheep		Goats	
	n/N*	(%)	n/N	(%)
<i>Signs:</i>				
- Fever	13/13	(100)	11/11	(100)
- Anorexia	12/13	(92.3)	11/11	(100)
- Diarrhea	11/13	(84.6)	11/11	(100)
- Naso-ocular discharge	11/13	(84.6)	10/11	(90.9)
- Dyspnea	8/13	(61.5)	9/11	(81.8)
- Ulcerative stomatitis	9/13	(69.2)	6/11	(54.5)
- Coughing	3/13	(23.1)	1/11	(9.1)
- Oro-nasal encrustations	1/13	(7.7)	1/11	(9.1)
<i>Lesions:</i>				
- Pneumonia	10/11	(90.9)	9/10	(90)
- Enteritis	9/11	(81.8)	9/10	(90)
- Hemorrhagic enteritis	4/11	(36.4)	4/10	(40)
- Hemorrhages and necrosis in the abomasal mucosa	2/11	(18.2)	3/10	(30)
- Pleurisy	1/11	(9.1)	0/10	(0)

*n/N means number of animals with feature/number of clinical PPR-affected animals

Case fatality rate

Data tabulated in table (2) revealed that the case fatality rates of PPR in the Southern region were (15.4% and 24.2%), (40% and 52.7%) and (27.8% and 13.9%) in sheep and goats respectively in groups of animals aged 4, 6 and 9 months. While in the central region of the kingdom, the case fatalities were 9.1%, 18.4% and 23.3% among sheep.

Table 2: PPR-case fatalities in association of with age groups in sheep and goats with laboratory confirmed disease in SA between March and August 2004.

Location	Sheep			Goats		
	Age	No. of affected flocks	case fatality rate	Age	No. of affected flocks	case fatality rate
Southern region; (Asser, Beshia)	4 m	(1)	20/130 (15.4%)	4 m	(4)	30/124 (24.2%)
	6 m	(1)	20/50 (40%)	6 m	(5)	68/129 (52.7%)
	9 m	(2)	5/18 (27.8%)	9 m	(2)	5/36 (13.9%)
Central region; (Riyadh, Thadeq AlKoyae, Hauta Sedear)	4 m	(1)	1/11 (9.1%)			
	6 m	(4)	29/158 (18.4%)			
	9 m	(3)	33/148 (22.3%)			
	1 y	(1)	0/10 (0%)			

m: month y: year

Detection and isolation of PPRV

Detection of PPR virus antigen by both IFA and IC-ELISA was succeeded in 100% of the tissue specimens (mouth scraping, lung and spleen), which collected from clinically affected sheep and goats, while trails for virus isolation from the same samples in cell cultures were unsuccessful (Table 3).

Table 3: Detection of PPRV in pre and post mortem samples collected from diseased sheep and goats

Samples	PPRV-antigen detection procedures		PPRV isolation on tissue culture
	IFA	IC-ELISA	
Mouth scraping	7/7	7/7 (4.7-6)**	0/7
Lung	4/4	4/4 (4.9-5.6)	0/4
Spleen	5/5	5/5 (4.7-6.2)	0/5

IFA: immunofluorescent antibody technique

IC-ELISA: immuno-capture enzyme linked immunosorbent assay

** range of equivalent virus infectivity titer (\log_{10} TCID₅₀)

Serodiagnosis

Table (4) represented the data of serodiagnosis of PPR in 6 flocks of sheep and 10 flocks of goats in central and southern regions of

the kingdom, it indicated that, PPR-seropositive percentage ranges and mean values were 80%-100% (93.8%) and 46.7%-100% (76.6%) respectively in sera of clinical-PPR sheep and goats.

Table 4: Detection of PPRV-antibodies in sera of affected sheep and goats as tested by competitive ELISA

Animal species	No. of the tested flocks	PPRV-antibodies		
		+ve/tested samples	+ve %	Range of +ve %
Sheep	6	30/32	93.8	80-100
Goats	10	49/64	76.6	46.7-100

DISCUSSION

The present study recorded an outbreak of PPR among sheep and goats in the central and southern regions of the Kingdom of Saudi Arabia between April-August 2004. During this outbreak, typical clinical signs of acute PPR including, fever, anorexia, diarrhea, mucopurulent nasal and ocular discharges, dyspnea and necro-ulcerative stomatitis; with a relatively high case fatality rates in lambs and kids aged 4 to 9 months; and gross lesions of pneumonia and enteritis were regularly recorded in PPR-affected sheep and goats flocks. Fatal acute PPR in sheep and goats is characterized by fever followed quickly by anorexia with inflammation of the upper and lower alimentary and respiratory tracts resulting in rapid development of ulcero-necrotic stomatitis, rhinitis, bronchopneumonia and enteritis which exhibited in signs of profuse, mucopurulent nasal and ocular discharges, encrustation on nares, eyes and lips, dyspnea and diarrhea (Braide, 1981). An outbreak of PPR characterized by fever, ocular and nasal discharge, coughing and sneezing, oral necrosis, diarrhea, enteritis and pneumonia was occurred in Ethiopian goats (Roeder, *et. al.* 1994).

In this reported outbreak, as a result of absence of vaccination against PPR in the affected sheep and goat flocks, restriction of the incidence of an acute clinical cases of PPR with case fatality rates ranged between 9.1 – 52.7% in animals aged 4-9 months is likely reflects the presence of immunity against PPR in a high percentage of adults due to previous exposure to the circulating virus that logically led to the occurrence of maternal immunity against the disease in majority of youngs until the 3rd month after parturition. Absolutely no record of clinical PPR in lambs and kids less than 3 months of age in the affected

flocks. However, case fatality rates were generally correlated with the age of diseased animals, it recorded values of (9.1-24.2%) in animals of 4 months of age, (18.4-52.7%) in animals of 6 months of age and (13.9-27.8%) in animals of 9 months of age.

Age resistant to PPR in sheep and goats is absolutely not true but, in enzootic areas the percentage of sheep and goats with antibodies to PPR virus rises with age, and the risk of acute fatal PPR is clearly confined to animals less than 1 year of age. This result agree with the findings of many authors, who mentioned that the disease is most potent in animals aged less than 1 year and this probably due to field infection with some virulent strains of PPR hastened by some bacterial infections such as pneumonic pasterullosis and enterotoxogenic *E. coli* (Losos, 1986; Radostitis *et. al.*, 2000). This result is pointed out (1) PPR virus infection in regions of study appears to have been primarily through the continuous movement and contact between infected and susceptible animals, and (2) the importance of vaccination of lambs and kids against PPR in such regions at the 3rd month of age.

In this study both PPRV-antigen and antibody assays were used to confirm the diagnosis of the disease. Approximately, seven affected sheep and goats flocks were diagnosed by PPRV-antigen detection in mouth scraping, spleen and lung tissue specimens using indirect fluorescent antibody (IFA) test and immuno-capture ELISA (IC-ELISA), and 16 affected flocks of sheep and goats were sero-diagnosed by detection of PPRV-antibodies using competitive ELISA (C-ELISA).

Sixteen tissue specimens were confirmed to be PPRV-antigen positive by both IFA and IC-ELISA. The obtained results suggest that (1) IFA test is a sensitive and reliable to demonstrate the cell associated PPRV-antigen; (2) commercial IC-ELISA is sensitive to detect and quantitate cell free PPRV-antigen; and (3) distribution of PPRV is seems to be equal in spleen, lung and mouth scraping where the recorded virus antigen concentrations were equivalent to virus titers ranged between 4.7 and 6.2 log₁₀ TCID₅₀. Immunocapture ELISA and immunofluorescence with virus specific monoclonal antibodies were used for detection and identification of PPR virus during an epizootic of PPR in northern India (Nanda, *et. al.*, 1996).

On the opposite side, all tissue specimens subjected for PPRV isolation throughout one passage on primary cell cultures of ovine kidney and three passages on cell line cultures of vero cells were given negative results. There is no obvious explanation for the failure to isolate PPR virus than the possibility that it had lost viability during

transportation and keeping of the samples, whatever is the explanation, IC-ELISA is a very specific, sensitive and rapid test for screening of PPRV antigen in tissue specimens, it can detect a very low titer ($06 \log_{10} \text{TCID}_{50}$) of virus (Libeau, *et. al.* 1994).

Monoclonal antibody-based competitive ELISA has been developed and used by Libeau, *et. al.* (1995); for the specific measurement of antibodies to PPR virus in sera of sheep and goats. In this report, as no available tissue specimens were received, serodiagnosis of PPR was carried out in 16 affected-flocks of sheep and goats by C-ELISA test. PPRV antibodies were detected in (40.7-100%) of sera collected from diseased sheep and goats. These high percentages of positive serum samples to PPRV antibodies are questionable but there are some points of concern in such cases, (1) serum samples were collected from individuals of PPR-infection suspicions between stage of illness and convalescence; (2) weaning age of PPR maternal antibodies in sheep and goats; (3) early development of PPRV-antibodies in the affected animals, and (4) history of vaccination against the disease in the flocks of study.

In conclusion, PPR outbreak among sheep and goats flocks in central and southern regions of the Kingdom mostly attributed to the presence of enzootic virulent strains of PPR virus, which could have circulate between the infected and susceptible animals in flocks of sheep and goats with no actual national vaccination regime against the disease.

The present study recommended that:

- An obligatory vaccination program against PPR virus infection should be applied for all flocks of sheep and goats all over the country.
- Lambs and kids should be vaccinated at 3rd month of age, when the maternal antibodies decline.
- Legalization of the restriction of the animal movement should be applied for sheep and goats populations during the risk period of PPR in different localities of the country.
- Imported small ruminants should have been vaccinated against PPR at least 10 days before their shipping from the exporting countries using the standard homologous vaccines.

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