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Dept. of Animal Medicine,
Fac. Vet. Med., Assiut University.

AN OUTBREAK OF PESTE DES PETITS RUMINANTS (PPR) AT ASWAN PROVINCE, EGYPT EVALUATION OF SOME NOVEL TOOLS FOR DIAGNOSIS OF PPR

(With 2 Tables)

By

U.ABD EL-HAKIM

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وباء مرض طاعون المجترات الصغيرة فى محافظة أسوان بمصر تقييم بعض الوسائل الحديثة لتشخيص مرض طاعون المجترات الصغيرة

أسامه عبد الحكيم

فى نهاية شهر يونيو وبداية شهر يوليو ٢٠٠٥ ظهر وباء بين الماعزوالأغنام فى محافظة أسوان بمصر. هذا الوباء تميز بوجود أعراض تنفسية وعينية وهضمية. لتحديد سبب هذا الوباء تم استخدام مائة من الماعز ومائة من الأغنام فى هذه الدراسة ، بعض هذة الحيوانات (٧٠ من الماعز و٤٥ من الأغنام) كانت تعانى من أعراض مرضية بينما باقى الحيوانات (٣٠ من الماعز و٥٥ من الأغنام) كانت سليمة ظاهريا. تم فحص هذه الحيوانات إكلينيكيا وسيرولوجيا. الفحص السيرولوجى شمل إختبار الإليزا التنافسى السريع وإختبار تعادل الفيروس للتعرف على الأجسام المناعية الخاصة بالمرض المسبب لهذا الوباء. الفحص الإكلينيكى سجل حمى ، إفرازات أنفية وعينية، إسهال ،التهابات فى الفم والتهاب رئوى فى الحيوانات المصابة. فى نفس الوقت أظهر الفحص الإكلينيكى أن الأعراض فى الماعز كانت أشد من الأعراض فى الأغنام. بعض الحيوانات التى ثبت إصابتهابعد إجراء الفحص السيرولوجى كانت سليمة ظاهريا ولا تعانى من أى أعراض مرضية (١٣ من الماعز و٦ من الأغنام). ٨٣ من الماعز و٥١ من الأغنام المختبرة كانت إيجابية لاختبار الإليزا التنافسى السريع بينما سجل اختبار التعادل الفيروسى ٧٦ حالة ايجابية بين الماعز و٤٧ حالة ايجابية بين الأغنام .كل العينات التى أعطت نتائج ايجابية مع اختبار التعادل الفيروسى كانت ايجابية مع اختبار الإليزا التنافسى السريع. نتائج الفحص الإكلينيكى والسيرولوجى أثبتت أن سبب الوباء محل الدراسة كان فيروس طاعون المجترات الصغيرة الذى يسبب مرض طاعون المجترات الصغيرة فى الماعز والأغنام. هذه الدراسة أثبتت أن اختبار الإليزا التنافسى السريع واختبار التعادل الفيروسى تقنيتان حساستان لتشخيص مرض طاعون المجترات الصغيرة. لهذا وعلى أية حال يمكن التوصية باستعمال اختبار الإليزا التنافسى السريع لتشخيص مرض طاعون المجترات الصغيرة خاصة فى حالات الاشتباه فى حدوث وباء مرض طاعون المجترات الصغيرة حيث أوضحت الدراسة أنه أسرع وأكثر حساسية

من اختبار التعادل الفيروسى .هذه أول دراسة تتناول تشخيص وباء مرض طاعون المجترات الصغيرة باستخدام اختبار الإليزا التنافسى السريع كما أنها الأولى التى تقارن بين هذا الاختبار واختبار التعادل الفيروسى فى تشخيص مرض طاعون المجترات الصغيرة فى مصر.

SUMMARY

An outbreak among goats and sheep at Aswan province, Egypt was observed. This outbreak manifested clinically by respiratory, ocular and digestive signs . To identify the cause of this outbreak, 100 goat and 100 sheep were used in the study, some of these animals (70 goat and 45 sheep) were clinically abnormal while the rest of them (30 goat and 55 sheep) were apparently healthy. These animals examined clinically and serologically. Serological examinations included recent rapid competitive enzyme linked immunosorbent assay (c-ELISA) and virus neutralization test (VNT) in order to detect the antibodies of the suspected aetiological agent of the outbreak. Clinical examination recorded fever, mucopurulent nasal and ocular discharge,erosive stomatitis , diarrhea and pneumonia. Clinical examination proved that clinical signs were more severe in goats than sheep. Some infected animals (13 goat and 6 sheep), as showed from serological results, were apparently healthy. 83 examined goat and 51 sheep were positive to rapid c-ELISA while VNT recorded 76 positive goat and 47 sheep. All samples gave positive result with VNT were positive with rapid c-ELISA. Clinical findings and serological results proved that the cause of the oubreak was the virus which causes peste des petits ruminants (PPR) in small ruminants. This study proved that rapid c-ELISA and VNT are sensitive techniques in diagnosis of PPR. However, it could be considered that usage of rapid c-ELISA seems faster and more sensitive than VNT. This is the first study concerning diagnosis of PPR by using rapid c-ELISA and the first to compare between rapid c-ELISA and VNT in diagnosis of PPR in Egypt.

Key words: *Peste des petits ruminants(PPR), PPR virus, rapid c-ELISA, VNT, Goats, Sheep, Outbreak, Egypt*

INTRODUCTION

Peste des petits ruminants (PPR) was first described in West Africa in the 1940s. The virus has been circulating in parts of Africa for

several decades and in the Middle East and Southern Asia since 1993 (Libeau *et al.*,1994; Libeau *et al.*,1995; Dhar *et al.*, 2002).

PPR is a highly contagious disease of domestic and wild small ruminants. It seems to be the major constraint in the development of small ruminant production in areas where it is endemic (Diallo, 2003). It is an important viral disease causing great economic losses in Africa, the Middle East and Asia (Awa *et al.*, 2000; Mitra-Kaushik *et al.*, 2001; Choi *et al.*, 2005).

PPR is caused by Peste des petits ruminants virus (PPRV), a member of the genus *Morbilivirus* (Which includes measles, rinderpest canine distemper and phocine distemper) within the family *Paramyxoviridae*. The virus is enveloped and it is antigenically closely related to rinderpest virus, which causes similar but distinct disease in large ruminant. PPRV harbors two major surface glycoproteins, the haemagglutinin-neuraminidase and the fusion proteins (Diallo *et al.*, 1994; Von Messling *et al.*, 1999; Sinnathamby *et al.*, 2001; Galbraith *et al.*, 2002).

Recently, many forms of ELISA have been used in diagnosis of PPR. These forms includes antigen-capture ELISA (Abraham and Berhan,2001), a sandwitch ELISA (Singh *et al.*, 2004c; Singh *et al.*, 2004d) and competitive ELISA (Choi *et al.*, 2003; Singh *et al.*, 2004a). Due to their simplicity, high sensitivity, and economy, several competitive ELISA have been recognized as suitable systems for use for diagnosis and seroepidemiological surveillance (Choi *et al.*, 2005).

In Egypt, PPR virus was isolated from goats in an outbreak of the disease for the first time in 1990 (Ismail and House, 1990; Ismail *et al.*, 1990). Since that time, a little work concerning PPR in Egypt has been done. Therefore, the aims of the present study were fellow up and identification of the cause of this outbreak using recent technique (Rapid c-ELISA) as well as comparison between rapid c-ELISA and traditional VNT to select the most reliable and sensitive technique that suitable for diagnostic (specially during outbreaks) studies of PPR in Egypt.

MATERIALS and METHODS

Animals: 100 goat and 100 sheep (6 – 24 months) were used for this study, some of them (70 goat and 45 sheep) were suffered from abnormal clinical signs and the rest (30 goat and 55 sheep) were apparently healthy.

Blood: 5 ml blood was collected twice two weeks apart from each examined animal (except 9 goats that died during this work and we obtained only one sample from them) to obtain serum for rapid c-ELISA and VNT.

Clinical examination: Clinical examination of all investigated animals was performed three times weekly. Body temperature, respiratory and heart rates as well as pulse were recorded. Any abnormal clinical signs were reported.

Rapid competitive enzyme linked immunosorbent assay (Rapid c-ELISA):

A PPRV c-ELISA kit developed at Centre de cooperation International en Recherche Agronomique pour le Developppement (CIRAD-EMVT), Montpellier, France, a Food and Agriculture Organization reference laboratory for PPRV, was used in the present work. All procedures were carried out according to the instructions in the manual included with the kit and as described by Choi *et al.* (2003) and Choi *et al.* (2005) as following:

1- Measurement the optical density (OD) of monoclonal antibody (MAb) alone:

- ELISA plate coated with 25 µl of PPRV antigen was incubated with 25 µl of peroxidase-labeled Mab P-3H12 conjugate in blocking buffer (0.01M PBS, 5% skim milk, 0.05% Tween 20) for 30 min at 37°C.
- After the plate was washed (using automatic plate washer wellwash-4, Labsystems, A thermo Bioanalysis Company, Research Technology Division)it was incubated with 50 µl of o-phenylendiamine substrate for 10 min at room temperature.
- The colorimetric reaction was stopped by adding of 50µl of 1.25 sulfuric acid.
- The OD of each well was measured at a wave length of 492 nm using Multiscan (Labsystems, A thermo Bioanalysis Company, Research Technology Division)

2- Measurement of OD of tested seum-Mab mixture:

- Antigen coated plates were incubated with 50 µl of a mixture of equal volume of the Mab P3H12 conjugate (final dilution, 1:800 in blocking buffer) and the tested serum sample (final dilution, 1:20 in blocking buffer) for 30 min at 37°C.
- Wells A1, A2 ,A3, A4, A5 and A6 of the coated plates were used for strongly positive, weakly positive and negative control sera respectively.
- All sera including the serum controls were tested in duplicate.

- The OD of each well was measured.

3- Calculation of percent inhibition (PI):

The OD of each well contain mixture of MAb and tested serum was converted into PI induced by the competition between the MAb and tested serum antibodies by the following formula:

$$PI = 1 - \{\frac{\text{(OD of mixture of MAb and tested serum)}}{\text{OD of MAb alone}}\} \times 100$$

4- Interpretation of results:

- Wells with PI values ≥ 50 were considered positive
- Wells with PI values between 45 and 49 were considered suspected and retested
- Wells with PI values ‹ 45 were considered negative

Rapid c-ELISA was performed twice two weeks apart for each examined animals (except for 9 goats that died during the study and after performing the first c-ELISA only).

Virus neutralization test (VNT):

Microtiter VNT technique was used according to Mariner *et al.*, (1993) with some modifications according to Choi *et al.* (2005) to detect neutralizing antibodies in sera as follow:

- Prior to the test, all sera were heat inactivated at 56°C for 30 min
- Twofold deletion series was created and started with a 1:10 dilution
- 25 µl of each serum dilution was added in duplicate wells, wells A1-5 were left without serum and served as control.
- Incubation with 100 µl of tissue culture of PPRV at 37°C for 45 min, wells B1-5 were left without virus and served as control.
- 100 µl of Vero cells was added to each well.
- The plate was monitored for cytopathic effect of PPRV for 7 days.
- The VNT titer was determined as the highest dilution of serum that inhibited the cytopathic effect of the virus.
- Sera with VNT titers of ≤ 10 were considered negative.

RESULTS

Clinical examination:

Clinical examination of investigated animals revealed great variation in severity of clinical signs between infected goats and sheep, where it was more severe in goats than in sheep. The clinical signs recorded in goats were fever (observed in 65 goat and lasted within 5 days),mucopurulent nasal discharge (observed in 57 goat and persisted

for 8 days), ocular discharge (observed in 43 goat and lasted within 6 days), diarrhea (observed in 40 goat and continued for 5 days, diarrhea was bloody in 7 goats only), erosive stomatitis (observed in 39 goat and persisted for 10 days) and pneumonia (observed in 28 goat and disappeared after 4 days except 9 goats that died within 2 days). The clinical signs recorded in sheep were fever (observed in 31 sheep and lasted within 3 days), nasal discharge (observed in 15 goat and persist for 4 days), ocular discharge (observed in 11 sheep and lasted within 3 days), diarrhea (observed in 19 sheep and continued for 5 days) and stomatitis (observed in 6 sheep and persisted for 7 days). Results of clinical examination are summarized in Table (1).

Table 1: Results of clinical examination.

	Fever	Nasal Discharge	Ocular Discharge	Diarrhea	Stomatitis	Pneumonia
Goats	65	57	43	40	39	28
Sheep	31	15	11	19	6	–

Rapid c-ELISA:

(a) First examination: 83 goat and 51 sheep were positive.

(b) Second examination: 74 goat (because 9 goats were died before performing the second examination) and 51 sheep were positive.

VNT:

(a) First examination: 76 goat and 47 sheep were positive.

(b) Second examination: 67 goat and 47 sheep were positive.

Results of laboratory investigation are summarized in Table (2).

Table 2: Results of laboratory investigation.

	Rapid c-ELISA		VNT	
	1st Examination	2nd Examination	1st Examinatio	2nd Examination
Goats	83	74	76	67
Sheep	51	51	47	47

DISCUSSION

PPR is an acute febrile contagious viral disease of small ruminants that of economic importance in Africa and in the Middle East where the disease has been circulating since 1993 (Dhar *et al.*, 2002; Choi *et al.*, 2005). In the present work we reported PPR as a cause of an

outbrek among goats and sheep. The disease has been recorded in Egypt for the first time in 1990 (Ismail and House, 1990; Ismail *et al.*, 1990).

Clinical investigation of animals examined in this study showed that infected animals were suffered from mucopurulent nasal and ocular discharges, erosive stomatitis, diarrhea and pneumonia. At the same time, the clinical signs among infected goats were more severe than in sheep. The same results and observation were reported by Awa *et al.*, (2002); Diallo (2003); Singh *et al.* (2004a); Singh *et al.* (2004b) and Cam *et al.* (2005) who suggested that goats more susceptible to infection with PPR virus than sheep. Another explanation of difference of severity of the clinical signs between infected goats and sheep could be understood from the work of Rajak *et al.* (2005) who concluded that PPR virus has a severe immunosuppressive effects in infected goats and these effects did not observed among infected sheep. Immune suppression associated with morbillivirus infection occurred through inhibition of leukocyte proliferation, apoptosis of peripheral blood mononuclear cells, downregulation of CD46 and influence the morbidity and clinical form of the disease (Sareen *et al.*, 1998; Mondal *et al.*, 2001; Heaney *et al.*, 2002). The third explanation of this observation is the fact that maternal antibodies persist and protect lambs for time longer than that in kids (Awa *et al.*, 2002; Diallo *et al.*, 2002). The observed clinical signs reported in this study may be correlated with immunohistochemical and pathological findings reported by Kumar *et al.* (2004) and Toplu *et al.* (2004) who concluded that the most characteristic immunohistochemical and pathological findings observed in infected animals in three outbreaks of PPR were erosive stomatitis, catarrhal or fibrinous bronchopneumonia, and acute catarrhal enteritis.

To avoid any interaction between maternal antibodies and results of our study, all animals used and examined in this work selected with 6 months to 2 years old. Work of Awa *et al.* (2002) and Diallo *et al.* (2002) proved that maternal antibodies to PPR virus in young animals were detectable up to 5 months of age and fell below the protection threshold level at 3.5 and 4.5 months in lambs and kids respectively.

In the present work, we examined paired sera samples from each examined animal for detection of antibodies to PPRV using rapid c-ELISA. Singh *et al.* (2004a) concluded that using c-ELISA test paired sera samples provided a clear diagnosis of PPRV infection and they added that rapid c-ELISA could clearly separate infected population from uninfected in field sera.

VNT was used for diagnosis of PPR in our study. In the last few years, VNT has been used in diagnosis and in evaluation of other diagnostic test of PPR (Dhinakar *et al.*, 2000; Nahed *et al.*, 2004; Singh *et al.*, 2004a; Choi *et al.*, 2005).

No difference between results of first and second rapid c-ELISA, as well as between first and second VNT has been reported in the present work. The same result has been reported in the work of Choi *et al.* (2003); Singh *et al.* (2004a) and Choi *et al.* (2005).

Our results proved that rapid c-ELISA is faster and more sensitive than VNT as it could detect infection among 134 animal (83 goat and 51 sheep) while VNT detected 1nfection among 123 animal (76 goat and 47 sheep) only, so we recommend using rapid c-ELISA in diagnosis of PPR in Egypt. Renukaradhya *et al.* (2003) and Singh *et al.* (2004a) concluded that c-ELISA test could easily replace VNT for sero-surveillance, sero-monitoring, diagnosis from paired sera samples and titration of PPR virus antibodies. They added that c-ELISA is simple, fast, reliable, and inexpensive tool for diagnostic and epidemiological purposes, as well as in disease eradication programs.

In this work, clinical examination and serological investigation showed that some infected animals (13 goat and 6 sheep) were apparently healthy. We consider these animals the most dangerous source of infection and play very important role in transmission of PPR from animal to animal and from area to area, therefore, detection of these animals are very important in control of PPR. Diop *et al.* (2005) reported that PPR subclinical infections do occur, and have an epidemiological importance in PPR (Shaila *et al.*, 1996; Berhe *et al.*, 2003).

From the result of our work we can conclude that rapid c-ELISA is fast and very sensitive assay in diagnosis of PPR and it can detect both clinically ill and apparently healthy infected goat and sheep. Rapid c-ELISA standardized here can be employed for diagnosis of PPR directly from field samples and proved to be more sensitive than VNT. Therefore, we recommend using of this technique in diagnosis of PPR in goats and sheep especially during any suspicious outbreak of PPR as it fast and sensitive test and so enable us to start early control measures to PPR.

This is the first report using rapid c-ELISA for diagnosis of PPR and the first to compare between rapid c-ELISA and VNT in diagnosis of PPR in Egypt

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