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THE ROLE OF CAMELS IN DESSIMINATION OF PESTE DES PETITS RUMINANTS VIRUS AMONG SHEEP AND GOATS IN SAUDI ARABIA

(With 2 Tables and One Figure)

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دور الجمال فى نشر فيروس مرض طاعون المجترات الصغيرة فى الأغنام والماعز بالعربية السعودية

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لدراسة مرض طاعون المجترات الصغيرة فى الجمال ومعرفة الدور الذى تلعبه الجمال فى نشر الفيروس المسبب للمرض تم استخدام ٥٠ جمل ، ٥٠ من الماعز و ٥٠ من الأغنام على احتكاك مباشر بهذه الجمال. ولقد تم إجراء هذه الدراسة البحثية على حيوانات إحدى المزارع الحيوانية بالمملكة العربية السعودية خلال العام ٢٠٠٥/ ٢٠٠٦ م. هذه الحيوانات تم فحصها من الناحية الإكلينيكية والفيروسية (العزل الفيروسى) وباستخدام التكنولوجيا الحيوية (اختبار التفاعل التبلمرى المتسلسل). تم اجراء العزل الفيروسى واختبارالتفاعل التبلمرى المتسلسل مرتين يفصل بينهما شهر كما تم فحص الحيوانات إكلينيكيا ٣ مرات اسبوعيا. كل الحيوانات المستخدمة كانت سليمة من الناحية الإكلينيكية كما أظهر الفحص الإكلينيكى الأول الذى تم اجراءه عند بداية العمل. فى الفحص الفيروسى الأول تم عزل فيروس طاعون المجترات الصغيرة والتعرف عليه فى ٦ جمال بينما لم يتم عزل الفيروس فى أى من الماعز أو الأغنام. بعد اجراء اختبار التفاعل التبلمرى المتسلسل الأول تم التعرف على الحامض النووى لفيروس طاعون المجترات الصغيرة فى ١٠ جمال وكانت كل الماعز والأغنام المختبرة سلبية لهذا الاختبار. فى الفحص الفيروسى الثانى تم عزل فيروس طاعون المجترات الصغيرة والتعرف عليه فى ١١ جمل و٣٢ من الماعز ولم يتم عزل الفيروس من أى من الأغنام التى تم فحصها. أظهر اختبار التفاعل التبلمرى المتسلسل الثانى وجود الحامض النووى لفيروس طاعون المجترات الصغيرة فى ١٧جمل و ٣٥ من الماعز بينما كانت كل الأغنام التى تم فحصها سلبية لهذا الاختبار. الفحص الإكلينيكى أوضح ظهور أعراض اكلينيكية على ٢١ من الماعز بعد اسبوعين من احتكاكها بالجمال التى ثبت اصابتها بفيروس طاعون المجترات الصغيرة. تمثلت هذه الأعراض فىحمى،التهابات فى الفم، إفرازات عينية وأنفية، كحة، اسهال والتهاب رئوى بينما عانى ٤ جمالٍ من حمى، افرازات أنفية وكحة فقط. فى نفس الوقت كانت باقى الحيوانات التى تم التأكد من اصابتها بفيروس طاعون المجترات الصغيرة بعد اجراء العزل الفيروسى واختبار التفاعل التبلمرى المتسلسل (١٣ جمل و١٤ من الماعز) سليمة من الناحية الإكلينيكية. نتائج هذا العمل أثبتت أن الجمال

قابلة للإصابة بمرض طاعون المجترات الصغيرة كما أنها تلعب دور هام جدا فى نقل الفيروس المسبب للمرض خاصة أن معظم الجمال المصابة كانت سليمة ظاهريا وهذا هو أخطر صور المرض من الناحية الوبائية إلا أن هذا الدور لم يثبت فى الأغنام المستخدمة فى هذا العمل. كما أوضحت نتائج هذا العمل أن التفاعل التبلمرى المتسلسل أسرع وأشد حساسية من العزل الفيروسى فى تشخيص مرض طاعون المجترات الصغيرة فى الجمال والماعز لهذا نوصى باستخدامه فى تشخيص المرض وفى أى دراسة وبائية للمرض كما نوصى باستعماله فى فحص الجمال الواردة من السودان للتأكد من خلوها من مرض طاعون المجترات الصغيرة حتى يمكن السيطرة على المرض والتخلص منه فى مصر. هذه أول دراسة تتناول مرض طاعون المجترات الصغيرة فى الجمال فىالدول العربية والدور الذى تلعبه الجمال فى نشر الفيروس المسبب للمرض كما أننا نسجل هنا أول استعمال لاختبار التفاعل التبلمرى المتسلسل لتشخيص مرض طاعون المجترات الصغيرة فى الجمال بالإضافة إلى التعرف على فيروس طاعون المجترات الصغيرة النوع-٢ والنوع-٤ لأول مرة فى الجمال.

SUMMARY

To study peste des petits ruminants (PPR) in camels and investigate role played by camels in transmission of PPR virus,50 camel, 50 goat and 50 sheep in contact with camels were used in the study. The animals were belonged to a private animal farm in the Kingdom of Saudi Arabia. These animals were examined clinically, virologically (virus isolation, VI), and by using molecular biology-based technique (Reverse transcription- polymerase chain reaction, RT-PCR). All used animals were clinically healthy as shown by clinical examination performed at the beginning of this work. Clinical examination was done three times a week while VI and RT-PCR were performed twice one month apart. Clinical examination showed that 4 camels were suffered from fever, nasal discharge and cough while 21 goats were suffered from fever, stomatitis, nasal and ocular discharges, diarrhea and pneumonia after two weeks from contact with infected camels. In the first virus isolation, PPR virus has been isolated from 6 camels while no PPR virus could be detected in examined goats and sheep. After performing first RT-PCR, PPR viral nucleic acid was identified in 10 camels but all examined goats and sheep were negative for this technique. PPR virus was isolated from 11 camels and 32 goats while all examined sheep were negative in the second virus isolation. The second RT-PCR showed presence of PPR viral nucleic acid in 17 camel and 35 goat while no PPR viral nucleic acid could be detected in all examined sheep. Results of this work proved that camel is susceptible to PPR and infected camel can transmit PPR virus to other camels as well as camels plays a very important role

in dessimination of PPR virus to contact goats. Dangerous of this role increased by the fact that most infected camels were apparently healthy. This role not proved in sheep examined in this study. Also, our results showed that RT-PCR is faster and more sensitive than VI in diagnosis of PPR so, we recommend using of this technique in routine diagnosis of PPR and in any epidemiological studies concerning PPR as well as using this sensitive assay in examination of camels imported from Sudan to can control and eradicate this disease from Egypt.

Key words: *Peste des petits ruminants (PPR), PPR virus, Camel, Sheep, Goat, RT-PCR, Virus isolation*

INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious disease of small ruminants with high rates of morbidity and sometimes mortality (Raj *et al.*, 2003; Choi *et al.*, 2005a). Economically, it was the most important disease of small ruminants in Africa, Middle east and southwest Asia (Shaila *et al.*, 1996; Berhe *et al.*, 2003). The disease included in the list A of the international zoosanitary code and it is also a part of the FAO Empress programme (Subash *et al.*, 2000; Diallo, 2003). Species susceptible to PPR virus are goats and sheep but it can infect deer and camels (Al-Naeem *et al.*, 2000; Haroun *et al.*, 2002; Abraham *et al.*, 2005).

PPR was first described in the Ivory Coast of Africa in 1942 (Couacy-Hymann *et al.*, 2005). After that, the geographic distribution of PPR is expanding and now includes Northern Africa, Asia and Turkey (Ozkul *et al.*, 2002; Taylor *et al.*, 2002; Lundervold *et al.*, 2004; Cam *et al.*, 2005). The disease was reported for the first time in Egypt in 1990 (Ismail and House, 1990; Ismail *et al.*, 1990).

PPR is spreading from areas of endemicity in Africa and Asia to neighboring countries and has devastated the livestock industry. Countries free of the disease, especially countries or regions neighboring areas of endemicity, require extensive surveillance to prevent the introduction of the disease to naive animal population (Choi *et al.*, 2005b).

PPR is caused by an enveloped RNA virus known as PPR virus which belongs to the *Morbilivirus* genus in the family *Paramyxoviridae* (Yilma *et al.*, 2003). Other members of the genus Morbilivirus include rinderpest virus (RPV), measles virus (MV), canine distemper virus (CDV), phocine distemper virus (PDV) and dolphin morbilivirus (DMV)

(Choi *et al.*, 2005a). PPR virus is genetically classified into four distinct lineages (I, II, III and IV) on the basis of partial sequence analysis of the fusion (F) protein (Choi *et al.*, 2005a). Many strains of PPR virus are exist in Africa (Couacy-Hymann *et al.*, 2005)

Rapid detection of infected animals is very important for PPR controls. Severe cases in which animals show clinical signs in the field can easily be detected through clinical surveillance and the detection of antigen in clinical samples, while the diagnosis of PPR infection in subclinically infected animals can be achieved by serological surveillance. However, the tests prescribed for the detection of PPR antibodies are laborious and expensive (Brindha *et al.*, 2001; Couacy-Hymann *et al.*, 2002: Choi *et al.*, 2005b).

Full length genome sequences for PPRV is published and the complete nucleotide sequence of the large polymerase (L) protein was determined. The gene is 6643 nucleotides in length and encoded a polypeptide of 2183 amino acids (Mahapatra *et al.*, 2003;Baily *et al.*, 2005; Muthuchelvan *et al.*, 2005a; Muthuchelvan *et al.*, 2005b).

RT-PCR system has been developed that detects and differentiates PPR virus from other closely related morbillivirus of ruminants. The capability of RT-PCR to deal with a large number of samples at a time and its short turnout time may be better serve the needs of surveillance and control programs. (Couacy-Hymann *et al.*, 2002; Forsyth *et al.*, 2003)

Information regarding PPR in camels are scarce and role of camels in dessimination of PPR virus and transmission of the disease to goats and sheep are not investigated before, therefore, the aim of the present work was to investigate PPR virus in camels and the role of these animals to dessiminate the virus among sheep and goats. Also, our goal was continued to compare between conventional VI and recent RT-PCR in diagnostic techniques.

MATERIALS and METHODS

Animals: 50 camel (recently imported from Sudan), 50 goats and 50 sheep in contact with camels were used in the study. All used animals were with age ranged from 6 months to two years. Animals of the planned work were belonged to a private farm in Saudi Arabia.

Blood: 5/ml unclotted blood (using EDTA) was collected two times one month apart from each examined animal. These samples were used for extraction of RNA of the virus.

Nasal swab: One nasal swab collected two times one month apart from each examined animal for isolation of the virus.

Ocular swab: One ocular swab collected two times one month apart from each examined animal for isolation of the virus.

1- 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.

2- Virus isolation (VI)

PPR virus was isolated from nasal and ocular swabs using Vero cell grown in Minimum essential medium (MEM) with 1% bovine fetal serum (GIBCO, Grand Island ,NY) which maintained for 21 days at 37 °C. The cytopathic effect of the virus (formation of multinucleated giant cell) was evaluated daily. The virus was identified and differentiated from other viruses causing syncytia (bovine respiratory syncytial virus and parainfluenza-3 virus) by immunofluorescence assay using fluorescent PPR virus antibodies (FA) conjugate (GIBCO, Grand Island, NY). Isolation and confirmation of presence of PPR virus were performed according to Nanda *et al.,* (1996) with the modification of Choi *et al.,* (2005a). The isolation of PPR virus as well as the various techniques were carried out in central laboratory of Ministry of Agriculture at Jeddah, Saudi Arabia.

3- Reverse transcription polymerase chain reaction (RT-PCR)

(a) Synthetic oligonucleotide primers:

The primers for PPR virus (Pharmacia Biotech) were selected by comparison of published sequences of PPR virus (Mahapatra *et al.,* 2003; Muthuchelvan *et al.,* 2005a). Four primers were used in the present study. The details of these primers are listed in Table (1).

Table 1: Sequences and location of the oligonucleotide primers used for PCR amplification of PPR virus.

Primer	Sequence 5`-3`	Expected virus	Position
N-F1	GAGCTCATGGCGACTCTCCTCAAAAG	PPR-L1	1-262
N-R525	AACCATGGTCAGCTGAGGAGATCCTTGT	PPR-L2	1-525
N-R262	CGCCGGCGGAGTCCGGCTTCTACAATAT	PPR-L3	1-447
N-F405	CGCCGGCGCTTCGGACCCATTTGGGATC	PPR-L4	405-521

PPR-L1=PPR Lineage-I PPR-L2=PPR Lineage-II PPR-L3=PPR Lineage-III PPR-L4=PPR Lineage-IV

(b) RNA extraction:

(b) RNA extraction:

Total RNA extraction was done as follow. Briefly, purified leucocyets were mixed with 5ml of guanidinum solution (4 M guanidinium isothiocyanate, 25mM sodium citrate pH 7.0, 0.5%w/v sarcosyl, and 0.1 M 2-mercaptoethanol). Next, 0.5 ml of 2 M sodium acetate pH 4.0, 5 ml of phenol and 12 ml of chloroform/isoamyl alcohol (24:1) were sequentially added. The mixture was shaken for 10 seconds, cooled on ice for 15 min, and centrifuged at10 000 g for 20 min at 4 °C. The aqueous phase was removed, precipitated with an equal volume of isopropanol, and placed at –20 °C overnight. The precipitated RNA was pelleted by centrifugation at 10 000 g for 30 min at 4°C. The RNA pellet was dried and then resuspended in 25 µl of diethylpyrocarbonate (DEPC) treated water.

(c) Primer directed amplification:

This step was done using reagent supplied in a kit (Perkin Elmer Cetus Corp., Norwalk, CT) and a programmable DNA thermal cycler (Coy Laboratory products Inc., Ann Arbor, MI). First strand Complementary DNA (cDNA) synthesis was done using reverse transcriptase and random primers. The first strand reaction contained 1X RT buffer(250 mM Tris-Hcl, pH8.3, 375 mM KCl and 15 mM MgCl2), 10 mM dithiotheritol, 1 mM of each deoxynucleotide (dATP, dGTP, dTTP, dCTP), 20units of RNasin, 300 ng of random hexanucleotide, 200 units of moloney murine leukemia virus reverse transcriptase (MMLV-RT), and 8.5 µl of extracted PPRV RNA in a reaction volume of 20 µ. The reaction mixture was incubated at 37 °C for 1 h, heat inactivated at 75 °C for 10 mins, and 80 µl of the following reaction mixture was added: 1X PCR buffer contains (5oo mM Kcl, 100 mMTris-HCl, pH 8.3, 1.25 µM of each upstream and downstream primers, 2.5 units of Ampli Taq polymerase, and 2 mM of MgCl2). The reaction mixture was initially denaturated at 94 °C for 4 min followed by 30 cycles of reaction parameters: template denaturationm at 94°C for 1 min, primer annealing at 55 °C for 1.5 min and extension at 72°C for 3 min.An additional incubation was done at 72°C for 7 min to complete the extension of templates.

(d)Identification of PCR products:

Following amplification, 10 µl of PCR product was examined by 1% agarose gel electrophoresis in Tris acetate EDTA (TAE) buffer using a 1 Kb ladder as molecular weight standard. The gels were stained with ethidium bromide (0.5 µl / ml). The specificity of PCR were determined by observing the expected size of PCR product in the gel.

RT-PCR was performed according to Forsyth and Barrett (1995) with modification of Saravanan *et al.*, (2004). Various instruments and chemical reagents required for PCR – test were kindly supplied and done at a highly equipped laboratory in Ministry of Agriculture, Jeddah, Saudi Arabia.

RESULTS

1- Clinical examination

(a) Camels: All camels were clinically healthy at the beginning of the study. After one week 4 camels were suffered from fever, nasal discharge and cough while rest of examined camels were clinically healthy even those proved to be infected with PPR virus.

(b) Goats: All goats were clinically healthy at the beginning of the study. After two weeks from contact with infected camels 21 goats showed the following clinical signs: fever, stomatitis, nasal and ocular discharges, bloody diarrhea and pneumonia.

(c) Sheep: All examined sheep were healthy along the entire time of this study.

2- VI

(a) First examination: PPR virus was isolated from 6 camels while no PPR virus could be isolated from examined goats and sheep.

(b) Second examination: PPR virus was isolated from 11 camel and 32 goat while all examined sheep were negative for virus isolation.

3- RT-PCR

(a) First examination: PPR viral nucleic acid was detected in 10 camels, the positive samples were belong to PPR virus lineage I (8 samples) and PPR virus lineage II (2 samples). No PPR viral nucleic acid detected in examined goats and sheep.

(b) Second examination: PPR viral nucleic acid was detected in 17 camel, the positive samples were belong to PPR virus lineage I (12 sample), PPR virus lineage II (3 samples) and PPR virus lineage IV (2 samples). PPR viral nucleic acid was detected in 35 goat, all of them were belong PPR virus lineage I. No nucleic acid detected in examined sheep.

Results of VI and RT-PCR are summarized in Table (2), and results of RT-PCR are shown in Figure (1).

Table 2: Results of laboratory investigation of examined animals.

	VIRUS ISOLSTION (VI)		RT-PCR					
	1st exam.	2nd exam.	1st exam.			2nd exam.		
			L.I	L.II	L.IV	L. I	L. II	LIV
CAMELS	6	11	8	2	-	12	3	2
GOATS	-	32	-	-	-	35	-	-
SHEEP	-	-	-	-	-	-	-	-

L.I = PPR virus lineage I L.II = PPR virus lineage II L.IV = PPR virus lineage IV

FIG.1

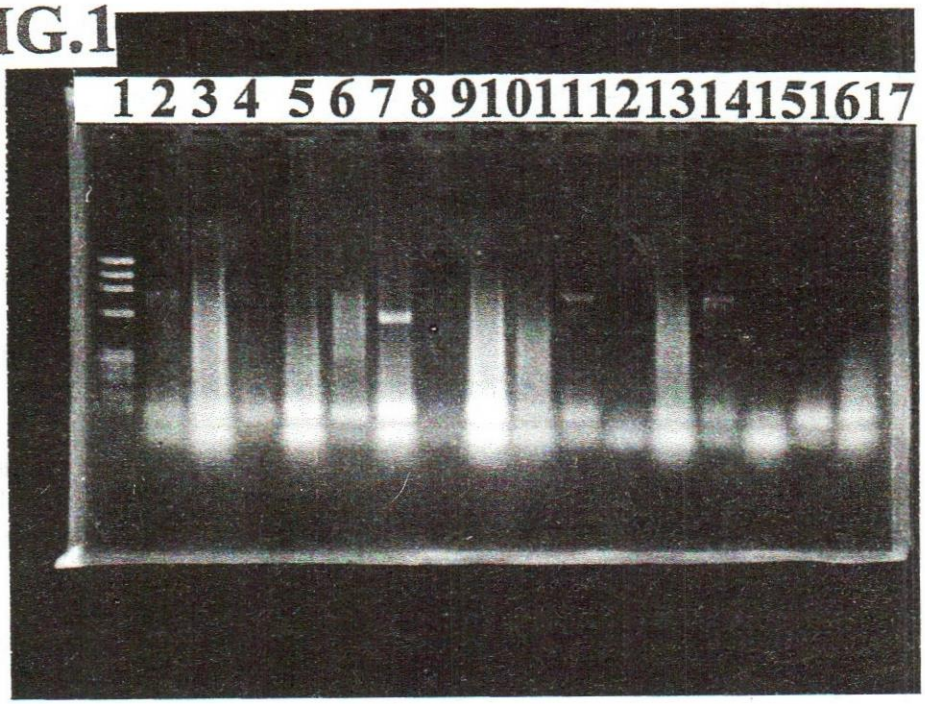

Fig. 1: Agarose gel electrophoresis of PCR product in examined camels, goats and sheep. Lane 1 (DNA ladder), Lane2 (PPRV-LineageI in camels), Lane6 (PPRV-LineageIV in camels), Lane7 (PPRV-LineageII in camels) Lanes 3, 4, 5 (negative results in camels), Lanes 11, 14 (PPRV-LineageI in goats), Lanes 8, 10, 12 (negative results in goats), Lanes 9, 13, 15, 16, 17 (negative results in sheep)

DISCUSSION

PPR is the most important disease of small ruminant in Africa and Middle East and recently it has become a much more prominent disease (Subash *et al.,* 2000; Berhe *et al.,* 2003).Goats and sheep are the species susceptible to PPR virus but the disease has been recorded in gazelles, deer, Arabian oryx and camels (El Zein *et al.,* 2004; Abraham *et al.,* 2005; Frolish *et al.,* 2005). In the present work we detected PPR virus in goats and camels. The disease has been reported in Egypt and most parts of Africa (Ismail *et al.,*1990; Abraham and Berhan, 2001). No virus could be detected in examined sheep. Roeder *et al.,* (1994) reported no affection among sheep during the first confirmed outbreak of PPR in Ethiopia and reported that goats are highly susceptible to PPR than sheep. The same observation has been recorded by Shaila *et al.,* (1996) who added that the most susceptible animals to infection with PPR virus are goats.

Clinical signs reported in infected animals in this work were more severe in goats than camels. The same result has been recorded by Haroun *et al.,* 2002, who concluded that goats are more susceptible to PPR virus than camels and usually suffered from acute form of the disease. However, most infected camels (13) and some infected goats (14) in our work were appeared healthy. Subclinical infection with PPR has been reported (Haroun *et al.,* 2002; Abraham *et al.,* 2005; Choi *et al.,* 2005b; Diop *et al.,* 2005) and only the most virulent strain show clinical disease (Subash *et al.,* 2000).

In this study we used nasal swabs for isolation of PPR virus. Diop *et al.,* (2005) in a survey carried out during PPR outbreak reported that between 85% and 100% of nasal secretions obtained from clinically diseased goats reacted positively to VI. We can add that PPR virus could be isolated from clinically diseased and apparently healthy goats and camels. At the same time, some clinically infected goats reacted negatively with VI in the present investigation. Animals with no detectable virus were able to seroconvert (Diop *et al.,* 2005).However, we isolated PPR virus from ocular swabs also. Ismail and House, (1990) isolated PPR virus for the first time in Egypt from the ocular secretions and Nanda *et al.,* (1996) and sumption *et al.,* (1998) concluded that PPR virus excreted in lachrymal secretions of infected animals. No difference reported in this study between isolation of PPR virus from nasal and ocular swab. Many authors isolated PPR virus from ocular and nasal secretions and reported no difference (Diop *et al.,* 2005).

In this work, we used whole blood for extraction of nucleic acid of PPR virus, Forsyth and Barrett (1995) and Saravanan *et al.*, (2004) used whole blood as a source of PPR nucleic acid. In our study we used RT-PCR for detection of nucleic acid of PPR virus. A highly sensitive technique (PCR) for the detection of PPR virus was developed (Mahapatra *et al.*, 2003; Saravanan *et al.*, 2004; Muthuchelvan *et al.*, 2005b)

RT-PCR used in this study detected PPR nucleic acid in some clinical samples which were negative by conventional VI. The same result was reported by Brindha *et al.*, (2001) and Saravanan *et al.* (2004) who added that RT-PCR technique was more sensitive than VI in detecting PPR virus in early as well as late phase of the disease.

In this work, all samples gave positive results with VI were positive with RT-PCR beside some samples gave positive results with RT-PCR only, therefore RT-PCR proved to be more sensitive than VI. In comparison with conventional techniques used in diagnosis of PPR, RT-PCR was more sensitive (Couacy-Hymann *et al.*, 2002; Forsyth *et al.*, 2003). The samples that gave positive results with RT-PCR were belong to PPR virus lineage I, lineage II and lineage IV in infected camels and belong to lineage I only in goats. Presence of more than one lineage of PPR virus has been recorded (Haroun *et al.*, 2002; Abraham *et al.*, 2005; Choi *et al.*, 2005a; Couacy-Hymann *et al.*, 2005)

Results of this study proved that PPR virus could be transmitted from infected camels to goats. In a work of Haroun *et al.*, (2002) and Abraham *et al.*, (2005), they detected seroprevalences in camels and goats and they concluded that this antibody seroprevalences confirmed natural transmission of PPR virus under field conditions. From our recent results reported in this work, we can add that detection of PPR virus and PPR viral nucleic acids in both camels and contact goats is a very strong evidence of transmission of PPR virus from infected camels to goats which were free from PPR virus and PPR viral nucleic acid at the beginning of this study and before contact with the camels.

From results of this work we can conclude that camels could be infected with PPR virus but they usually show no clinical signs of the disease, therefore, they play a very important role in dessimination of PPR virus to susceptible goats in contact with them but this role did not observe in sheep examined in this study. PPR virus could be isolated from ocular and nasal swabs of clinically diseased and apparently healthy animals. RT-PCR is more sensitive than VI and could be used when VI failed to detect PPR virus, so, we recommend using of this

sensitive technique for diagnosis of PPR and for routine examination of imported camel in order to control and eradicate this dangerous disease from Egypt. This is the first study concerning PPR in camels in Arabic countries and role played by camels in dessimination of PPR virus and we recorded here the first using of RT-PCR in diagnosis of PPR in camels, in addition to the first recognition of PPR virus lineage II and lineage IV in camels.

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