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Virological Diagnoses of Pesti des petitis Ruminants Virus in Sheep in Giza Governorate

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PESTE des petits ruminants (PPR) is an intense and extremely contagious disease with high mortality in small ruminants resulting inconsiderable socio-economic impact in developing countries. Our study was established during the routine follow up in El-Giza governorate during 2019. A total number of 45 swabs, 40 sera samples and 15 post-mortem epithelial tissues were collected from sheep showing clinical signs suggestive for PPR.PPRVdetection was investigated using serological, pathological and RT-PCRdiagnosis tests.Serologically, PPRV antigens detection were 86%, 93.3%, 66.63% and 73.3% in the nasal, ocular, faecal swabs and different tissues samples, respectively. While, PPRV antibodies detection were 95% in the collected sera samples.Histopathologically, the lung showed severe bronchiectasis and syncytium formation with high accumulation of inflammatory cells. The liver exhibited necrosis with severe portal and central hypertension filled with inflammatory cells.The kidneys revealed severe atrophied glomeruli with destructed renal glomerular capillaries, hemorrhage in the glomeruli and renal tubules with thrombosis of blood vessels. Spleen revealed proliferation of central arterioles with periarteriolar proliferation of connective tissues and hypercellular red pulp with hemorrhage. The mouth commissures suffering from nodules and ulceration which exhibited microscopically multiple vesicles in the epidermis and dissociation of the epidermal and dermal layers filled with inflammatory cells and eosinophils with syncytium formation. A 448 bp band by RT-PCRwas obtained from all nasal and ocular samples, while 2 lung samples were negative for this test.Therefore, the results proved the presence of the disease in El-Giza governorate.

Keywords: Peste des petits ruminants, Sheep, ELISA, Histopathology, RT-PCR.

Introduction

Peste des Petits Ruminants (PPR) is anacute contagious small ruminant's disease with socioeconomic impact of10-90% and 50-90% morbidity and mortality rates, respectively, in naive populations [1]. Hence, it affects thesustainable farmers livelihood and food security across Africa, the Middle East and Asia [2]. The etiological agent of the disease is the Peste des petits ruminants virus (PPRV) belonging to Morbillivirus genus, sub-family Paramyxovirinae, family Paramyxoviridae, and order Mononegavirales [3].

The disease, characterized by pyrexia (up to 41°C) lasting for 3-5 days, depression, anorexia,muzzle dryness,watery lachrymal and nasal discharges andnecrotic erosive lesions in the oral cavitythat may progress in severe cases

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and be accompanied by fibrin deposition (caseous deposit) on the tongue.In later stages of the disease, diarrhea,labored coughing and abdominal breathingsymptoms are developed todyspneic, progressive weight loss and emaciation, and finally death of the animal.In mild cases, animals may convalesce, within 10-15 days, returning to a pre-infection health status. In the acute form of disease, morbidity and fatality rate may reach 100% [4,5]. The variation in clinical signs and mortality depends on the viral strain virulently and the immunological status of the infected animals [6]. As the virus concentration is high in oral and lacrimal secretions, the infection is easily transmitted orally or respiratory or via direct contact between infected and susceptible animals [7].

According to the global eradication program of the Food and Agricultural Organization of the United Nations (FAO) and the World Animal Health Organization (OIE), Egypt is attempting to control and eradicate the disease until 2030, in this way usingroutine specific diagnostic tests that known to be simpler, rapid, robust and user-friendly helped unquestionably to improve and simplify the official PPR control strategies (vaccination campaigns) throughout the country. Hence, effective PPR screening depends mainly on a proper and a rapid diagnosis.

As the clinical differentiation between PPRV and other morbilli viruses like rinderpest (RP) is difficult and they are unstable outside their living host, the laboratory confirmation of suspected cases is necessary.Virus isolation in cell culture is considered one of the most definitive diagnosis used [8] but is still time consumed as it need 10 -12 days for virus isolation.Rapid, common and specific sero-monitoring of the disease antigens and/ or antibodies within a large number of samples can be done nowadays by commercial diagnostic kits;ELISAs tests; thatused indetection of either the N or the H proteinsof PPRV [9].

As PPR disease consider a hurdle to sustainable agriculture development through Africa, using recent confirmatory molecular tools in PPRdiagnosis were proved to be sensitive and specific to detect the genome of PPR virus [10] and opened a new way of disease investigation to be recommended by OIE.Mainly, two standard PCRs, Reverse transcription (RT-PCR) and quantitative real-time RT-PCR have been developed to amplify parts of the fusion (surface glycoprotein), or the nucleoprotein (most abundant internal protein), or the matrix protein genes[11], thus helps in genetically typing the PPRV isolates from different outbreaks and then subsequent assessment of the epidemiological lineage distribution in endemic areas of Africa, thus leading to speculating the dominant lineage emergence of PPRV in new areas [11].Genetically, the Egyptian isolates have been identified as belonging to lineage IV,and this lineage is becoming the predominant lineage in Africa, suggesting that perhaps Egypt is the gateway for the predominantly PPRV Asian lineageentry into Africa [12,13].

In Egypt, the first outbreak of PPR befell was in January 1987 amongst goats at Kafr Hakim, Embaba and Giza Governorate, where fatality rate reached 30%. PPR virus was isolated on Vero cells and then recognized with direct fluorescent antibody method (DFAT) [14]. Mouaz [15] isolated PPR virus from infected animals in Giza governorate and have been identified as PPR Giza 94. Mahmoud et al.[16]concluded that the Seroprevalence antibodies analysis against foot and mouth disease (FMD), Peste des Petits ruminants (PPR) and bluetongue (BT), revealed that Giza and Beni-Suef governorates are endemic for these viruses in sheep and goats. Also, Nafea et al.[17]concluded that PPRV is still circulating insome Egyptgovernorates (Sharkia, Kafr El-sheikh, Marsa-Matrouh, Giza, Sharkiya and Red sea),leading to outbreaks in its major host,especially small ruminants.

The present study aimed to highlight on the epidemiological situation of PPR infection in El-Giza governorate through the laboratory detection of PPR viral antigen and antibodies by ELISA tests, histopathological examination then using Reverse Transcription-Polymerase Reaction (RT-PCR) as a final confirmation step.

Materials and Methods

Ethical approval

The experiments were carried out in accordance with the guidelines laid down by the National Research Centre, Institutional Animal Ethics Committee and in accordance with local laws and regulations.

Sample collection, transportation and preparation

A total of one hundred animal clinical samples (swabs and blood) were collected from apparently healthy and diseased sheep, while tissue samples were post-mortemcollected from two recently dead and one emergency slaughtered sheep.

A-Swabs: nasal (n=15), ocular (n=15) and Faecal (n=15) swabs were aseptically collected and then suspended in 2 ml of 0.9% saline solution, homogenized, centrifuged at 2000 rpm for 10 min at 4 °C, the supernatant was aliquoted and stored at -20°C until used for PPRV antigens and molecular analysis [18].

B- Blood samples: 40blood samples were collected aseptically from the jugular vein from each animal using plain vacutainer tubes without anticoagulant. Serum was separated by centrifugation of the blood at 3000 rpm for 10 min at room temperature; aliquots were transferred into a 1.5 ml sterile microcentrifuge tube. All serum samples were stored at -20°C until used for a PPRV antibodies investigation [16].

C- Epithelial tissues: A total of 15 tissues samples (necrotic tissue of mouth, lung, liver, spleen, kidney, intestine and intestinal lymph node) were collected then carried on ice tothe laboratory for further analysis. Each specimen was divided into 3 portions, one placed in 10% neutral buffered formalin [19], the second was preserved at -80° C and the third portion was preserved in equal amounts of glycerol and 0.04 M PBS, grounded, centrifuged at approximately 2000 rpm for 10 minutes to remove cell debris and the supernatant was used undiluted for PPRV pathological, molecular and serological examinations, respectively.

Laboratory diagnosis

Serological tests

A-Detection of PPRV antigens by Immunocapture ELISA

Immunocapture ELISA kit for detection of PPRV antigen in the tissue samples and swabs jointly produced by BDSL (biological diagnostic supplies Ltd) flow laboratories and CIRAD EMVT Montbellier, France; the test was carried according to the instruction of the manual included with the kit as described by Mahmoud et al. [16]

B-Detection of PPRV antibodies by competitive ELISA

The procedure was carried according to the instruction of the manual of PPR Competition ELISA diagnostic kit manufactured by ID Screen, Vet Innovative diagnostics, France. The wells are coated with purified recombinant PPR nucleo protein (NP). Anti NP antibodies, if present, form an antibody-antigen complex which masks the NP epitopes. An anti-NP peroxidase conjugate is added to the micro wells. It fixes to the remaining free NP epitopes, forming an antigen- conjugate – peroxidase complex. After adding the substrate solution, the resulting coloration depends on the quantity of specific antibodies present in the samples to be tested [20]. Calculation of the mean OD value of the positive control (OD posC) < 30% of the OD of the negative control. OD of NgC is greater than 0.7. Sample representing a S/N :- less than or equal 50% are considered positive-greater than 50% and less than or equal 60% are considered doubtful- greater than 60% are considered negative.

Histopathological examination

The fixed tissue specimens were kept in formalin for at least 24 hours and then routinely processed by conventional method and finally stained by Heamatoxylene and Eosin for the detection of the histopathological changes [19].

RT-PCR detection of PPR viruses

A- Extraction and quantification of viral RNA

Viral RNA was extracted from 250 µl of all nasal, ocular swabs and lung tissues using TRIZOL (cat #15596-026, Invtrogen, Germany) method according to the instructions provided by the manufacturer. 1µl of the final eluted Viral RNA from each sample was used for the determination of RNA concentration and purity using a NanoDrop (Thermo Scientific, Waltham, MA, USA). The value A260/A280 = 1.8 or above were considered as pure and used in RT-PCR detection of PPR viruses and then kept at -80°C for further molecular experiments.

B- Reverse Transcription-Polymerase reaction (RT-PCR)

Reverse transcription (RT) and partial amplification of fusion protein gene of PPR virus was performed using a single tube RT-PCR method "One-step RT-PCR Kit" protocol (SuperScript® III Reverse Transcriptase- Invitrogen) following the manufacturer's instruction.A 448-bp fragment of PPR-F gene was amplified using forward (F1b) (5'-AGTACAAAAGATTGCTGATCACAGT) and reverse (F2d) (5'- GGGTCTCGAAGGCTAG GCCCGAATA) primers set according to OIE [36]. A total of 35 cycle of PCR amplification was carried out in a thermocycler (Bio-Rad, USA) with an initial denaturation at 94°c for 2 min. The subsequent cycling condition consisting of denaturation at 94°c for 1 min, annealing at 55°C for 1 min , extension at 72°C for 1 min and final extension at 72°C for 7 min. The reaction was hold at 4°C, the PCR amplicons were electrophoresed on 1.5% agarose gel containing ethidium

bromide and images were captured using a Gel documention system (Bio-Rad, USA).

Results

Clinical signs

All animals in the flock appeared infected with 69% morbidity rate and 31% mortality rate. The animals showed emaciation, depression and dehydration. The body temperature ranged from 40-41ºC. Profuse diarrhoea, coughing,sneezing, difficult breathing associated with nasal and lacrimal discharges. Some animals showed swelling eye lids. Most animals showed severe congestion in the mucous membranes of the gum and tongue as well as necrotic lesions on the lips (Fig. 1a,b).

Virological studies

A-Detection of PPRV antigens by Immunocapture ELISA

Detection of PPRV antigen by IC-ELISA in swabs and tissues (Table1) revealed that out of 15 nasal,ocular, Faecal swabs and tissues 13, 14, 10 and 11 samples are positive, respectively.

B-Detection of PPRV antibodies by competitive ELISA

The data for serological diagnosis of PPRV antibodies in the collected serum samples from Giza Governorate was represented in Table 1, revealed that out of 40 serum samples 38 samples were positive.

C- Histopathological evaluation

The macroscopic appearance of organs revealed severe consolidated pale lung (Fig. 2a),thickened inflamed intestine with nodular thickening (Fig. 2b) and the mouth commissure revealed ulceration and vesicles formation (Fig. 2c).

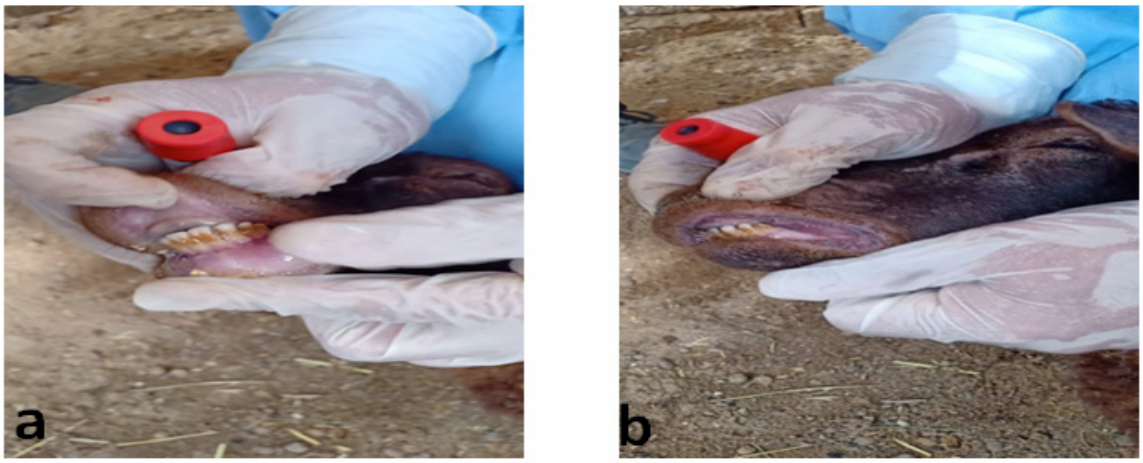

Fig. 1. Clinical signs of naturally infected sheep with PPRV.a) Necrotic ulcer on the lip of sheep. b) Necrotic tissue on gum and tongue.

TABLE 1. Detection of PPR antigen/ antibodies in the different samples

Test	Samples	+Ve	-Ve	% of +Ve
IC-ELISA (Antigen detection)	Nasal swabs (n=15)	13	2	86%
	Ocular swabs (n=15)	14	1	93.3%
	Faecal swab (n=15)	10	5	66.6%
	Tissues (n=15)	11	4	73.3%
	Total (n=60)	48	12	80%
C-ELISA (Antibodies detection)	Serum samples (n=40)	38	2	95%

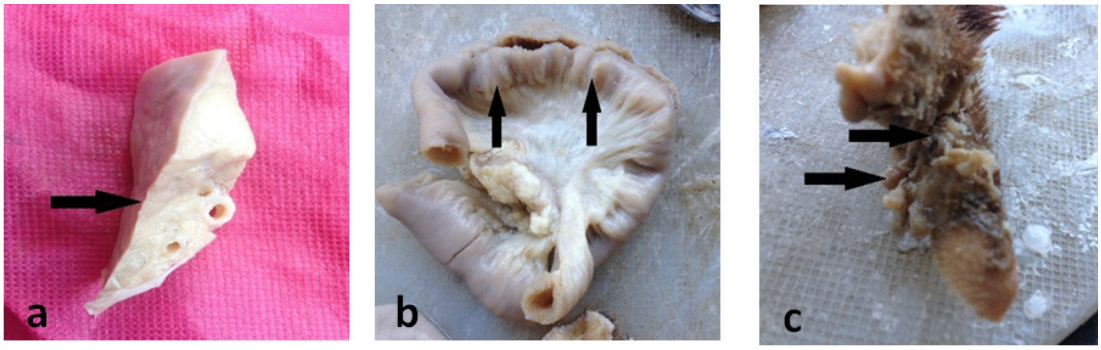

Fig. 2. Gross pathological changes in various organs of PPR infected sheep. a)Lung showing sever consolidation and compressed tissue with pale in color (arrow). b) Intestine showing thickened wall with thickened nodules and change in color (arrows). C) Mouth commissure showing vesicles and ulcerations and change in color (arrows).

The microscopic appearance of lung revealed severe atelectasis,dilated bronchioles filled with necrotic and inflammatory cells and compressed by interstitial proliferated connective tissues (Fig. 3a).The lung alveoli filled with sloughed necrosed tissues and inflammatory cells (Fig. 3b)the degenerated cells (neutrophils and macrophages) appeared filling the alveoli (Fig. 3c),the bronchioles revealed severely dilatation and proliferation of epithelium with folding into lumen (Fig. 3d), the bronchioles showed severely dilated proliferated metaplastic epithelium from into pseudostratified epithelium proliferated into lumen (Fig. 3e). The bronchiolar epithelium have degenerative changes with syncetial cells formation in the wall of bronchioles and in the lumen (Fig. 3f).

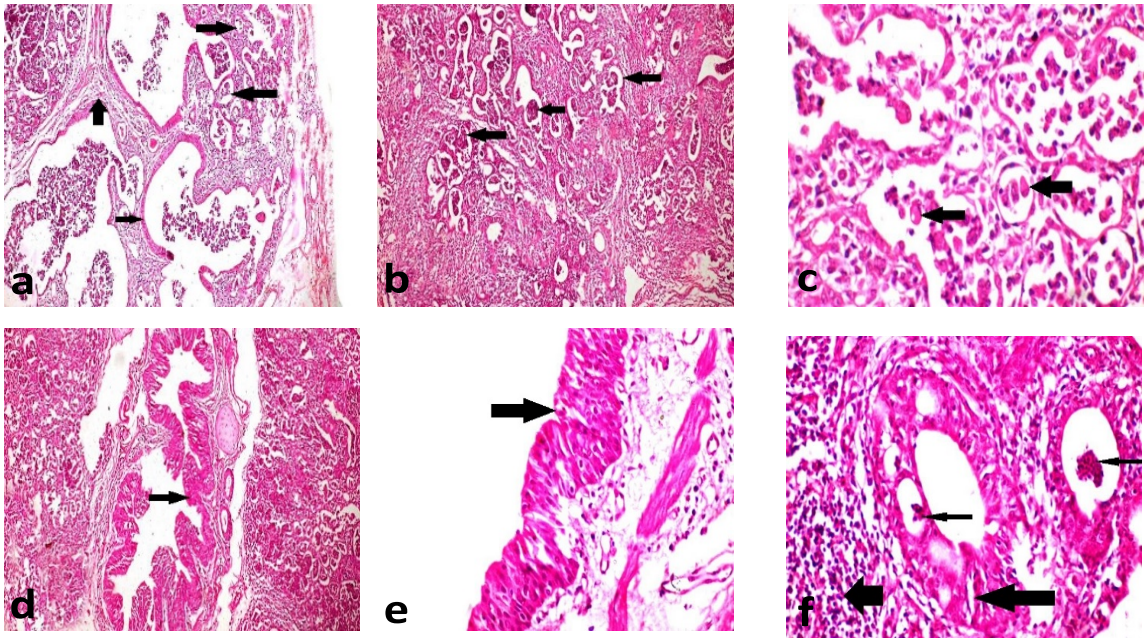

Fig. 3. Histopathological changes in lung of PPR infected sheep revealing a)Atelectatic lung alveoli (long arrow) with severely dilated bronchioles filled with necrotic tissues and inflammatory cells (short thin arrow) with fibrosis of the interstitial tissues (short thick arrow) (H&EX100).b) Compressed alveoli filled with necrotic and inflammatory cells (arrows) the interstitial tissues exhibiting fibrosis (H&EX200). c) Alveoli filled with cells have eosinophilic intracytoplasmic inclusion (arrows) (H&EX600). d) Severely dilated proliferated epithelium of bronchioles with folding into lumen (arrow) (H&EX100). e) High power of the bronchioles showing severe hyperplasia and metaplasia of the bronchiolar columnar epithelium into pseudostratified epithelium (arrow) (H&Ex400). f) Hyperplasia of bronchiolar epithelium (long thick arrow) with scattered epithelium degeneration forming syncytial cells in the wall and in lumen (long thin arrow) with severe infiltration of mononuclear cells (short arrow) (H&EX400).

The liver showed hepatocytes with necrotic changes, pyknotic nuclei with small shrinked ones, some nuclei fade off (Fig. 4a),intracytoplasmic vacuolated hepatocytes (intracytoplasmic vacuoles) push the nuclei into periphery with margination of cytoplasm with necrotic changes in hepatocytes (Fig. 4b), the central vein congested filled with inflammatory cells and the portal vein also congested and areas of dissociated dissolved hepatocytes (Fig. 4c).

The kidneys showed severe degeneration and necrosis of glomerular blood capillaries with atrophied glomeruli (Fig. 5a)with complete loss of glomeruli **(**Fig. 5b),hemorrhage with hemosiderin pigments present in glomeruli (Fig. 5c), the renal tubules showed degenerative changes, thickening in the wall of blood vessels of kidneys with thrombus formation(Fig. 5d)and severe hemorrhage in renal tubuli (Fig. 5e).

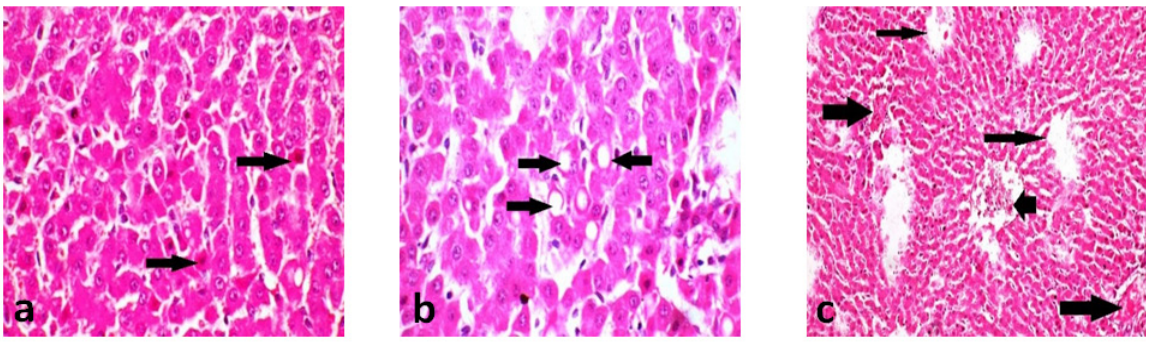

Fig. 4. Histopathological changes in liver of PPR infected sheep showinga) Some necrotic hepatocytes changes, pyknotic and shrinked nuclei, some nuclei fade off (arrows) (H&EX200).b) Intracytoplasmic vacuoles pushing the nuclei into periphery with margination of cytoplasm (H&EX400).c) Severe dissociated and necrotic hepatocytes (long thin arrows), congested central vein filled with inflammatory cells (short thick arrow), and congested portal vein filled with blood (long thick arrow) (H&EX100).

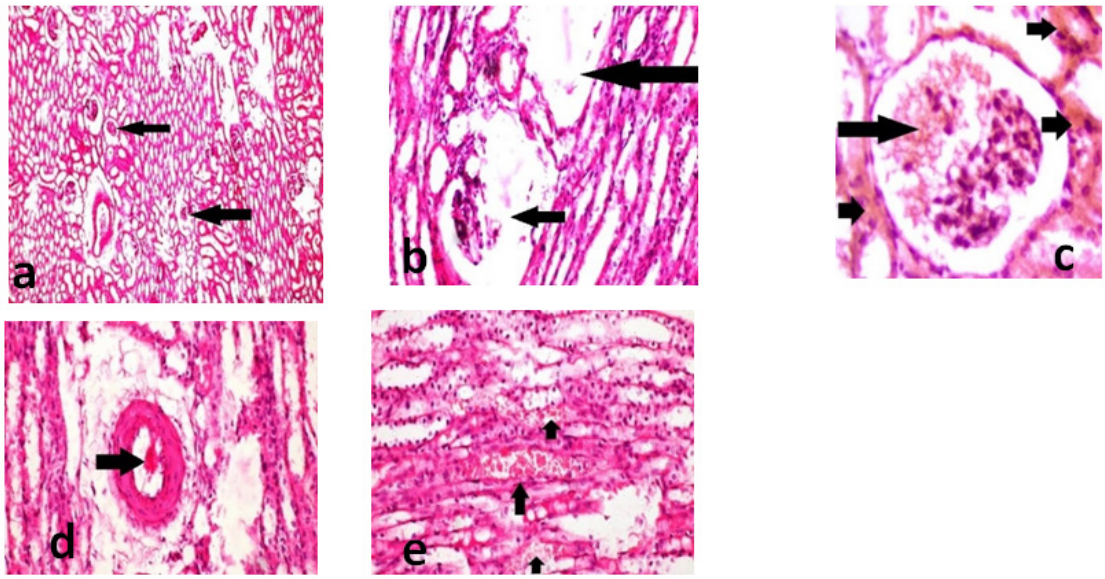

Fig. 5. Histopathological changes in kidneys of PPR infected sheep exhibiting a) Atrophied and shrinkage renal glomeruli with increase glomerular space (arrows) (H&EX100). b) High power kidneys showing complete loss of renal glomeruli and empty glomerular space (long arrow) atrophied destructed glomeruli and highly widened space (short arrow)(H&EX400). c) Renal glomeruli filled with Rbcs and hemorrhagic casts (long arrow) with atrophied glomeruli and hemorrhage extend to renal tubuli (short arrows, H&EX600). d) Renal blood vessels with thrombosis in the lumen (arrow) (H&EX400). e) Hemorrhage in the renal tubuli (arrows) (H&EX400).

The intestine showed severe sloughing of the intestinal villi with blunting and stunting of intestinal villi (Fig. 6a),destruction of payer's patches, atrophied of the follicles with edema in the tunica media (Fig. 6b), depletion of lymphoid follicles and apoptosis of lymphocytes (Fig. 6c), intranuclear inclusion bodies in upper epithelial of intestinal villi and thrombosis in the blood capillaries supply of tunica media (Fig. 6e).

The spleen revealed proliferation of central arterioles showing (Fig.7a) periarteriolar proliferation of fibrous connective tissue as onion shape appearance and hypercellular red pulp and hemorrhage(Fig. 7b)and suffered from hemorrhage and hemosiderin pigments (Fig. 7c).

The skin of mouth commissure suffered from pseudomembrane formation vesicles in the epidermal layer of skin,the stratum spinosum suffered from degenerative changes and formation of syncytial cells (Fig. 8a,b) andsome parts of epidermis and dermis revealed complete lysis and degeneration with accumulation of degenerated neutrophils and macrophages (Fig. 8c).

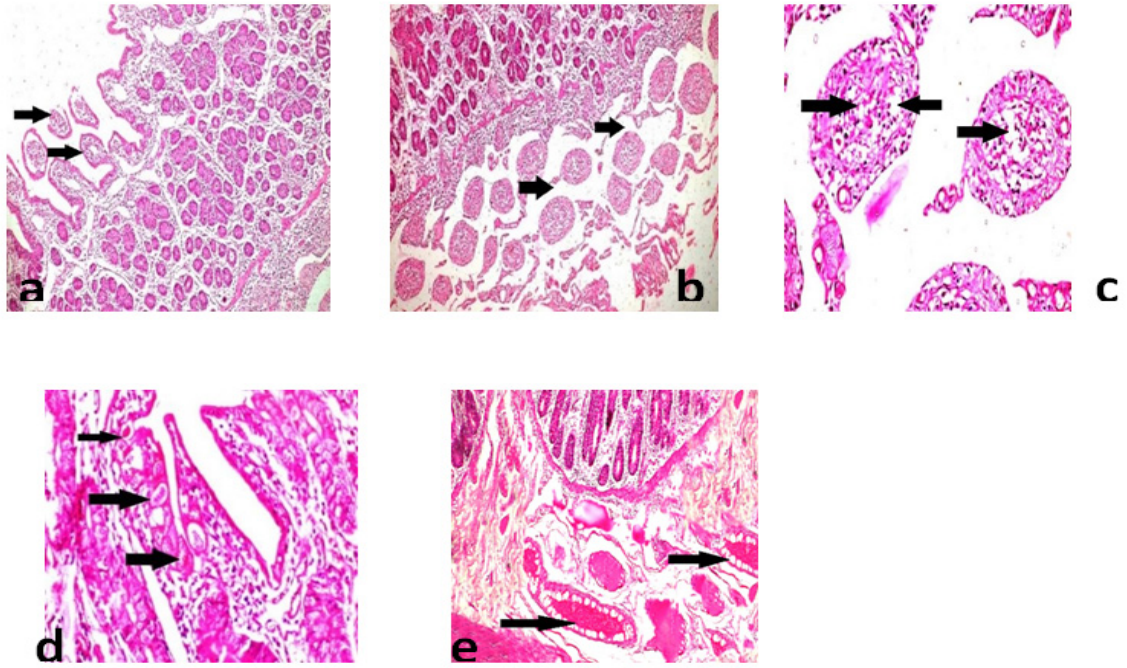

Fig. 6. Histopathological changes in intestine of PPR infected sheep revealing a) Necrotic and sloughed Intestinal villi epithelium and blunting of some (arrows) (H&EX200). b) Destructed and atrophied Intestinal payer's patches with edema in the tunica media (arrows) (H&EX200). c) High power showing severe depletion of lymphoid follicles and apoptosis of lymphocytes (arrows) (H&EX400). d) Intestinal epithelium with eosinophilic intracytoplasmic inclusions (arrows) (H&EX600). e) Intestine showing thrombosis in intestinal blood vessels (arrows) (H&EX200).

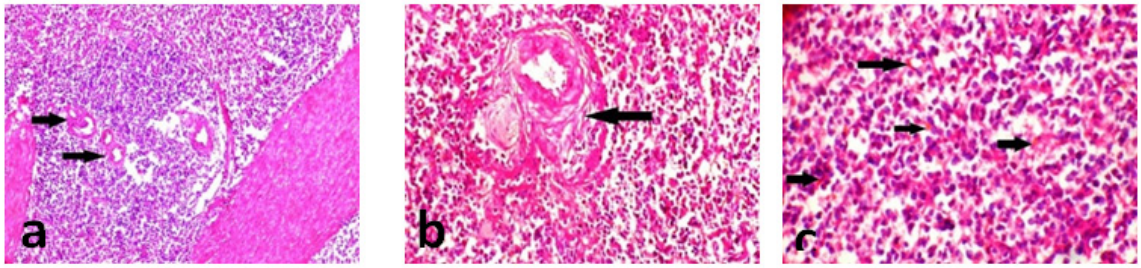

Fig. 7. Histopathological changes in spleen of PPR infected sheep showing a) Proliferation of central arteriole (arrows)(H&EX100). b) Periarteriolar proliferation of fibrous connective tissue (arrow) and hypercellular red pulp with hemorrhage (H&EX200). c) Hemorrhage (arrows) (H&EX400).

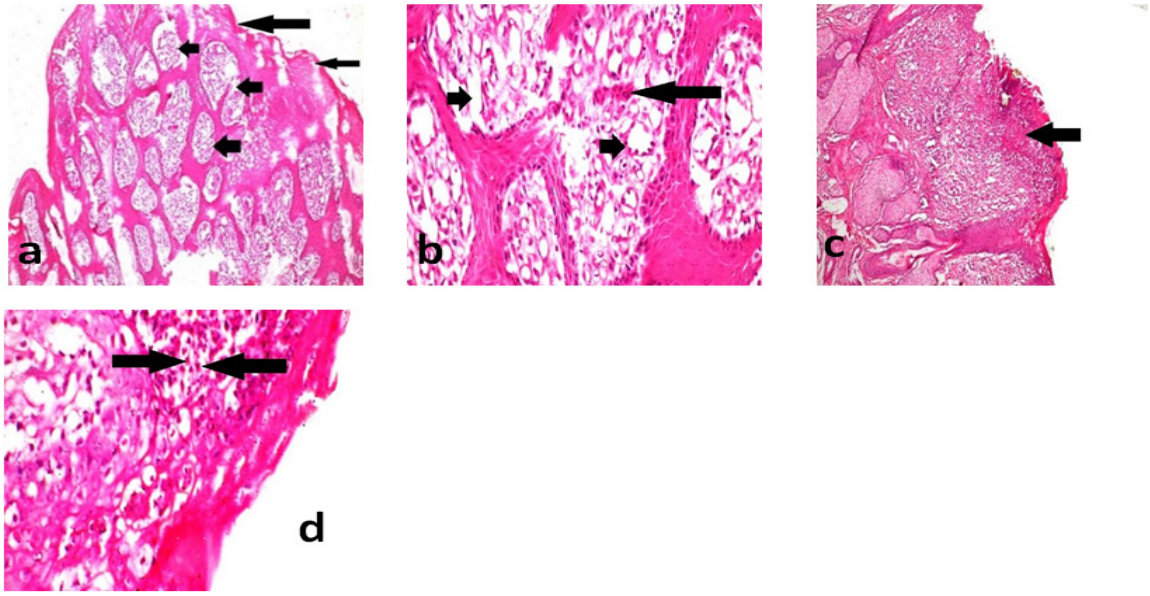

Fig. 8. Histopathological changes in skinmouth commissureof PPR infected sheep revealing a) Pseudo membrane (long arrow) and multiple vesicles in skin epidermis (H&EX100). b) High power of the vesicles showing degenerated stratum spinosum cells forming vacuoles with syncytial formation inside the vacuoles (short arrow) and syncytial cells formation out the vacuoles (long arrow)(H&EX400). c) Skin showing degeneration and necrosis with lysis of epidermal and dermal layer filled with inflammatory cells (arrow) (H&EX100). d) High power of previous picture showing accumulation of neutrophils in the degenerated skin (arrow) (H&EX600).

D- Molecular evaluation

In the present study,the nasal and ocular swabs (n = 27) from seropositive animals and lung samples (n=3) were further tested for the PPRV confirmation using reverse transcription-polymerase chain reaction (RT-PCR).

Among the samples analyzed in this study, 28 gave successful amplified product showed 448-bp fragment (Figure9). This viral nucleic acid was detected in nasal and ocular swabs, in one lung sampleand in the positive control (PC); while No viral RNA was detected in the othertwo tested lung samples and in the used negative control (NC, with no RNA templates).

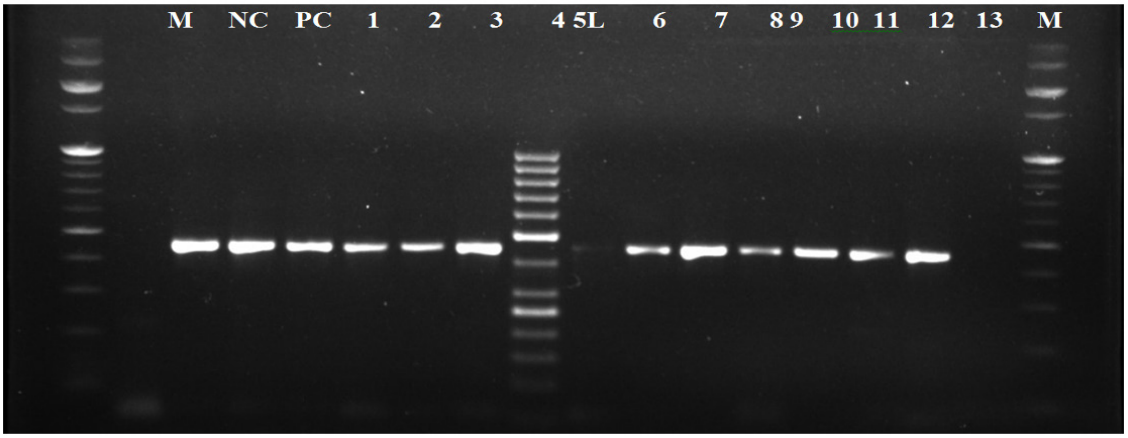

Fig. 9. Agarose gel electrophoresis of F gene (448 bp) of PPR virus. Lane M: 100 bp DNA ladder marker (Intron Scientific); Lane NC: Negative control; Lane PC: Positive control; Lane L: 50 bp DNA ladder marker (50,100,150, 200, 250, 300, 400, 500, theromo Scientific); lanes 1-5 and 7-12: positive PPR of nasal and ocular samples; respectively and Lanes 6, 13: positive, negative PPR samples collected from Lung, respectively.

Discussion

Peste des petits ruminants (PPR) disease is important disease affecting sheep and goats and produce severe economic losses among them. The present study recorded reappearance of the disease during (2019) in Giza governorate. Typical signs of acute PPR disease including fever, anorexia, diarrhea, mucopurulent nasal and ocular discharge, cough, erosion and ulcer around mouth, gross lesions of pneumonia and enteritis with varying erosion degrees in the gut mucosa were recorded. The liver and kidney were congested. These results were agreed with some authors[6,16].

Different methods are used for diagnosis and monitoring the PPRV distribution and prevalence, such as: SNT, AGID, FAT and PCR, etc.In the current study, diagnosis and confirmation of PPRV were done by using ELISA, RT-PCR and histopathology examination. Due to cross reaction between PPRV and RPV, monoclonal antibody based C-ELISA was developed for PPR diagnosis andconsideredas a highly sensitive and specific assay than virus isolation and became more preferable and alternative to it [20].In this study, PPRV antigens were detected in swabs and tissue samples using IC-ELISA. The results revealed thatswabsand tissue samples were positive by percentage of 82.2% (37/45) and 73.3% (11/15), respectively, this result agreed with the study of Abd El-Rahim et al.[21] whotested nasal and ocular swabs for PPR antigensdetection using IC-ELISA and found that PPR antigens were detected in 30/40 (75%) of the swabs.

The collected sera samples in this study were examined by competitive ELISA. The results of PPR antibodies detection showed that 38 out of 40 samples (95%) were positive and these positive results agreed with Hosny et al. [2] study, in which the positivity for PPR antibodies were found in 60 out of 62 sera samples by percentage of(96.7%). Also, Abubakar et al. [22]found that84 out of110 sera samples were tested positive for PPR antibodies by C-ELISA. Saeed et al. [23] tested sheep and goat sera samples for PPRV antibodies and obtained 80%positivity by using the previous test.Mahmoud and Galbat [24] explained that, these highly obtained seropositive percentages could be related to many factors such as young age, low maternal immunity intake, poor nutritional status and drastic climatic conditions, which in turn increase the animals' susceptibility to PPR infection.

Histopathology of the lung samples revealed that, the severe changes that recorded in alveoli and bronchioles with syncytial cells formation and eosinophilic intracytoplasmic and intranuclear inclusion bodies were in agreement with kumar et al. [25] who observed various degrees of interstitial changes in bronchioles, alveolar macrophages and syncytial cells had intracytoplasmic and intranuclear eosinophilic inclusions and these changes in 57% of the studied animals were linked with serofibrinous pneumonia. Also, Kul et al. [26] recorded broncho-interstital pneumonia in sheep and goat lungs infected by PPR. Parida et al. [3]recorded many alveolar macrophages, mononuclear cells with interalveolar septa thickness and intranuclear eosinophilic inclusion bodies. Begum et al. [27] found that the infected lungs with PPR virus showed clumps of large mononuclear and multinucleated cells within the alveoli andPrajapati et al. [28]observed fibrino-necrotic broncho-interstitial pneumonia and syncytial formation in pulmonary alveoli in sheep and goat infected with PPR virus. These multinucleated enlarged cells (syncytia) were formed from fusion of the infected cells with their neighboring cells and this process is induced by viral fusion protein expression at the surface of the host cell membrane and this finding is supported by Shahriari et al. [29] who found similar cytopathic effect (CPE) between PPR and *Morbillivirus* infection, in which rounding cells aggregated, syncytia formed and cells destruction occurred after twelve days of PI of the infection.

Necrosis in liver was supported by Sahinduran et al. [30], while Khan et al. [31] found congestion in liver and degeneration of hepatocytes in goat infected with PPR. The changes in spleen and depletion of white pulp were supported by kumar et al. [24] who found necrosis in spleen. Begum et al. [27] observed hemosiderosis with severe congestion in the spleen. Fayyad et al. [32] found lymphoid organs damage, peripheral blood mononuclear cells apoptosis, and presence of many necrotic lesions in the spleen, lymph nodes, thymus and Peyer›s patches. The proliferation of central arterioles in spleen supported by Zhu et al. [33], who foundhigh venous pressure and hemodynamics that can result in vascular proliferation of penicillar arterioles of spleen, lymphoplasia and increase venous pressure related to portal hypertension.

Kidneys lesions recorded were in agreement with kul et al. [26] who found immunoglobulins

of PPR in glomeruli and also Begum et al. [27] reported hemorrhage in the tubular area and severe hemorrhages, congestion with hemosiderosis in kidneys of infected sheep by PPR infection. Hemorrhagic areas and necrotic foci were seen in livers and kidneys supported by Sahinduran et al. [30].

The observed ulcerative in skin of mouth commissure was in agreement with Kumar et al. [25] who found degeneration and necrosis of labial mucosa and concluded that PPRV is both lympho- and epithelio-tropic. Kul et al. [26] and Sahinduran et al. [30] found erosive ulcerative stomatitis in sheep and goat infected by PPR.

Intestinal lesions observed in our study were in agreement with Kumar et al. [25] who found necrosis in payer's patches of intestine. Squamous epithelial syncytial formation, necrotic lesions in the intestinal lymph nodes, degeneration and necrosis of intestinal epithelium and lymphoid cell depletion from Peyer›s patches.

Also, Parida et al.[3] observed blunting and stunting villi, necrotic enteritis and infiltration of mononuclear cells small intestines exhibited diffuse edema of the submucosa along with proliferation of fibrocytes leading to thickened submucosa. Lymphoid organs showed partial to complete destruction of lymphoid follicles.

Thrombosis recorded in kidneys and intestine were supported by Ugochukwu etal. [34] who found that PPR disease increase disseminated intravascular coagulopathy.

In our study, only seropositive samples swabs (nasal, n=13; ocular, n=14) and three post-mortem samples from lung tissues were tested for diagnosis of PPRV infection using RT-PCR targeting the F gene and we found that, all swabs samples and one lung tissue tested positive, while the other two lung tissues were tested negative for the presence of F gene fragment (448bp)[35]. Many previous studies used the same primer sequences to detect F protein (coding region) for PPR virus from different clinical samples [36,26].

Depending on Manzoor et al. [37]base rule'sto select a suitable diagnostic test; in which the onewith at least 95% sensitivity and 75% specificity is preferable to rule out a disease, while the test with at least 95% specificity and 75% sensitivity is used to confirm the disease.In our study, the seropositive samples swabs (nasal, n=13; ocular, n=14)that analysed by IC-ELISA and further tested through one-step RT-PCR showed quite homology (100%) between them. This findings are in line with that ofManzoor et al. [37]who obtained a good agreement between both tests. This result is also agreed with OIE manual, in which both tests are considered the most widely used assays.

The molecular techniques offer many advantages, but unfortunately their results critically depend on the genetic composition of the virus, quality of field samples, the type of host, age of the susceptible animalsand species or breeds variations.

For diagnosis of PPRV infection, non-invasive field samples taken from live infected animals like ocular discharges, nasal secretions, mouth and gum erosions samples, rectal mucosae, feacal samples and anticoagulant-treated blood provide a valuable source of viral RNA, are easily obtained, easily transported to the laboratory and are ideal for endemic countries since mortality rates are still low and postmortem tissue samples may be not available yet. While lymph nodes (mesenteric and bronchial), spleen, brain, lungs, skin and intestinal mucosae obtained from necropsied animals are recommended [38,39]. Controversial results obtained by many authors, ŞEVİK[٣٩] obtained positive results in all samples of liver, lung, lymph nodes and spleen using conventional and real time RT-PCR targeting the F and N gene, respectively, and he concluded that these samples can equally be used for viral RNAdetection using these assays. While, Albayrak and Alkan [40] found that the maximum positive percentage rate were 50, 22.58 and 17 in tissue samples of lymph nodes, spleen and lungs, respectively, while this percentage were 25 and 10 in nasal and conjunctival swab samples, respectively, whereas blood and oral swab samples were negative for PPRV by RT-PCR.Also, KUL et al., [26] conducted RT-PCR to detect PPRV and pestivirus in different tissue samples, collected from lung, lymph node, brain, spleen and skin and found that spleen and lymph node were positive, while lung, brain and skin tissues were tested negative.While Forsyth and Barett [41] used RT-PCR targeting F gene to confirm the disease in nasal, rectal swabs, lung and mesenteric lymph nodes tissues and found that only mesenteric lymph node sample was positive. On the other hand, using blood in virus detection tests, depends mainly on the sampling time (viraemic phase), but the failure chance for PPRV detection is still high [41].

Conclusion

The present study recorded reappearance of the disease during 2019 and this result is confirmed by the used diagnostic tests understudy. In order to obtain a complete epidemiological picture of the circulating PPR strains across the government, continues monitoring through different investigations studiesare needed,proper diagnosis thatuse reliable and rapid toolsshould be adopted, thus finally helps in developing efficient control programs at the official level to eradicate this disease in Egypt.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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التشخيص الفيرولوجى لفيروس مرض طاعون المجترات الصغيرة في الأغنام فى محافظة الجيزة

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يعتبر طاعون المجترات الصغيرة مرض شديد ومعدٍ للغاية،ويتميز بارتفاع معدل الوفيات في تلك الحيوانات،والتى تؤثر بالتبعية على الناحية الاجتماعية والاقتصادية خاصة في البلدان النامية. أثناء الدراسة الحالية ومن خلال المتابعة الروتينية في محافظة الجيزة خلال عام ٢٠١٩. تم جمع عدد إجمالي ٤٥ مسحة و ٤٠ عينة مصل من الدم و ١٥ عينة نسيجيةمختلفة من الأغنام المذبوحة والتي تظهر عليها علامات سريرية توحي بالإصابةبطاعون المجترات الصغيرة. من حيث الاختبارات السيرلوجية ، كان اكتشاف مستضد PPRV ٦٨٪ و ٩٣,٣٪ و ٦٦,٦٣٪ و ٧٣,٣٪ في عينات مسحات الأنف والعين والبراز وعينات الأنسجة المختلفة على التوالي. بينما بلغت نسبة اكتشاف الأجسام المضادة للـ PPRV في عينات المصل التي تم جمعها ٩٥٪. من الناحية الهستولوجيه ، أظهرت الرئة توسع القصبات الحاد وتكوين المخلوي مع تراكم عالٍ للخلايا الالتهابية. أظهر الكبد نخرًا مصحوبًا بارتفاع شديد في الضغط البابي وضغط الدم المركزي مليئًا بالخلايا الالتهابية. كشفت الكلى عن الكبيبات الضامرة الشديدة مع الشعيرات الدموية الكبيبية الكلوية المدمرة ، ونزيف في الكبيبات والأنابيب الكلوية مع تجلط الأوعية الدموية. كشف الطحال عن تكاثر الشرايين المركزية مع تكاثر حول الشريان للأنسجة الضامة واللب الأحمر فوق الخلايا مع النزف.أظهرت مفاصل الفم من عقيدات وتقرحات تظهر حويصلات متعددة مجهريًا في البشرة وتفكك طبقات البشرة والأدمة المليئة بالخلايا الالتهابية والحمضات مع تكوين المخلوقات. كما تم إجراء اختبار تفاعل البوليميراز المتسلسل RT-PCR للكشف عن جين بروتين الاندماج لفيروس طاعون المجترات الصغيرة. تم الحصول على حزمة عند ٤٤٨ bp من جميع عينات الأنف والعين ، بينما كانت عينات الرئة سالبة لهذا الاختبار.

الكلمات الدالة: طاعون المجترات الصغيرة، الأغنام، االأليزا، الفحص التشريحى، RT-PCR