Histopathological and Immunohistochemical Studies on The Female Genital System and Some Visceral Organs in Sheep and Goat Naturally Infected by Peste Des Petits Ruminants Virus

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Abstract: The aim of study is describing the pathological changes in the genital system and some visceral organs of sheep and goat naturally infected with Peste des Petits Ruminants (PPR) disease. A number of 17 sheep and 15 goats aged from 2 to 4 years were used which showing a clinical signs as profuse watery diarrhea with dark brown coloration, acute respiratory distress and of low conception rate; these cases were slaughtered and then undergo postmortem examination for natural PPR virus infection. The Pathological findings were found in genital system included lymphocytic; ulcerative and hemorrhagic endometritis. In other visceral organs found Lymphocytic enteritis accompanied with coagglutative necrosis in the whole length of intestinal villi; bronchointerstitial lymphocytic pneumonia accompanied with severe degeneration and necrosis of bronchial epithelium, also perirenal multifocal lymphocytic infiltration; vacuolar degeneration in the liver. These pathological finding were confirmed by detection of antibodies against PPR virus in serum of the suspected cases by using C-ELISA and also by using immunohistochemistry the viral antigen was detected in the uterus, lung and small intestine. In conclusions the PPR virus can be gain access to the reproductive system causing ulcerative lymphocytic endometritis and affect in the reproductive performance of sheep and goats.

Key words: Immunopathology • Peste virus • Small ruminants • Reproduction

INTRODUCTION

Peste des Petits Ruminants (PPR) is currently considered as one of the main animal transboundary viral diseases that constitute a threat to livestock production in many developing countries [1]. PPR virus has a widespread distribution spanning West and Central Africa, the Middle East and southern Asia [2, 3]. PPR virus is highly contagious and easily transmitted by direct contact between the secretions and/or excretions of infected animals and nearby healthy animals [4]. The disease constitutes one of the major obstacles to subsistence farming; mortality from infection reaching 50-80% in a naïve population [5].

The disease is the fourth member of Morbillivirus, which belongs to the family Paramyxoviridae and has a close relationship with rinder pest, measles, canine distemper and phocine distemper viruses [6, 7]. Structurally, the morbilliviruses are morphologically pleomorphic particles (400-500 nm) similar in appearance to other members of the family Paramyxoviridae being enveloped (cell membrane derived) with viral glycoproteins seen as plomers protruding from the envelope. Under the electron microscope the negative-sense RNA genome in association with viral protein is also visible; this ribonucleoprotein (RNP) complex forms a helical structure and in appearance resembles a ‘herring bone’ [8]. The nonsegmented, negative-strand genome of PPRV encodes eight proteins: the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F) and the haemagglutinin protein (H), [9].

Co-infection with both PPR virus and pestiviruses in cases of abortion has been reported in lambs [10]. Histopathological findings resulting from PPR virus infection include pseudomembranous, erosive and ulcerative stomatitis; necrotic tonsillitis;
fibrinohaemorrhagic enteritis; and bronchointerstitial pneumonia. [11-13]. Among the characteristic histopathological findings of PPRV infection are syncytial cells in affected oral mucosa and lungs, as well as eosinophilic nuclear and cytoplasmic inclusion bodies, especially in the respiratory and/or alimentary tract epithelia. [13-15].

The C-ELISA was developed for detection of antibodies to PPR virus in serum samples of goats and sheep. The test used monoclonal antibody to neutralizing the epitope of haemaglutinin protein of the virus. Efficacy of C-ELISA compared very well with VNT, having high relative specificity (98.4%) and sensitivity (92.4%). The sensitivity of C-ELISA for PPR virus sero-surveillance was (95.4%) if the target population was non-vaccinated. It was opined that the C-ELISA developed could easily replace VNT for sero-surveillance, seromonitoring diagnosis from paired sera samples and end-point titration of PPR virus antibodies [16]. The virus has also, albeit rarely, been linked to infertility and abortion in infected animals. The importance of this and the mechanisms by which it occurs are currently unknown [1]. The objectives of this study were to describe the histopathological, immunohistochemical changes in the female reproductive system of sheep and goats due to natural PPR virus infection; also the histopathological changes in some of visceral organs as intestine, lungs and liver which affect on the general health condition.

### MATERIALS AND METHODS

**Animals:** The blood and tissue samples for this study were collected during slaughtering and post mortem examination of ewes (17 cases) and goats (15 cases) aged from 2 to 4 years which marked by the farm serial numbers as recorded in table (1) submitted from different flocks exhibiting clinical signs, such as respiratory distress, diarrhea, lowering of conception rates. And had a history of no vaccination against PPR virus.

**Competitive ELISA:** Blood samples were collected from the jugular veins in a clean unheparinized labeled tubes then centrifuged at 3000 revolutions per minute for 5 minutes for separation of serum then stored in deep freezer at -20ºC until C-ELISA were done. ID screen PPR competition ELISA kit for the detection of antibodies against the PPRV in sheep and goat serum and plasma by competitive screening was used, this diagnostic kit is designed to detect antibodies directed against the nucleoprotein of the PPR virus. A commercial C-ELISA kit; used to detect sero-positive animal; was applied according to manufacturerÔs instructions (ID-Vet innovative diagnostic, ID Screen® PPR competition) C-ELISA were developed according to [17]. The test uses technology developed by a FAO reference Laboratory (CIRAD- EMVT, Montpellier, France) and all procedures were performed according to the instruction manual.

<table>
<thead>
<tr>
<th>Species</th>
<th>Case No.</th>
<th>C-ELISA Results</th>
<th>Uterus</th>
<th>Lung</th>
<th>Intestine</th>
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</table>

Immunohistochemistry scoring + mild ++ moderate +++ intense. Case No. according to farm records.
The wells are coated with purified recombinant PPR nucleoprotein (NP) the samples to be tested and controls are added to the micro wells. Anti-NP antibodies, if present, form an antibody-antigen complex. 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 that washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested, in the absence of antibodies; a blue solution appears which becomes yellow after addition of the stop solution, in the presence of antibodies, no coloration sample. Then calculated according to the equation:

\[
\text{Competition} \% = \frac{\text{Optic density (OD) sample}}{\text{Optic density (OD) negative control}} \times 100.
\]

The sample considered positive if the competition % less than or equal to 50%.

**Histopathology:** For histopathology examination; tissue samples of female reproductive system as uterus, ovary and oviduct, as well as lung, intestine and liver were fixed in 10% neutral buffer formalin for 24 to 48 hours, routinely processed, embedded in paraffin wax and sectioned at 5 µm [18].

**Immunohistochemistry:** The tissue samples collected for immunohistochemistry examination; were transformed to this study. Alcohol 70% after 24 hr. to protect the antigenisity of PPR virus from formalin fixative. A commercial streptavidin/biotin immunoperoxidase kit ((EconoTek Horse radish peroxidase (HRP). Diaminobenzedine stain (DAB) Anti-Polyvalent. Scy Tek lab.)) was used and all procedures were done according to Haines & Clark. [19] and performed according to the instruction manual. Tissue sections were digested with Proteinase K (0.1%) and incubated with goat anti Peste des Petits Ruminants antibody imported from FAO reference Laboratory (CIRAD- EMVT, Montpellier, France) at a dilution of 1/100. Diaminobenzidine chromogen substrate system was applied for color reaction. Peste des Petits Ruminants virus positive lung tissues, previously confirmed with PCR, were used as positive controls. Lung sections from sheep free from PPR virus infection were used as negative controls. Immunoperoxidase scoring was made on the basis of positively stained cells observed in 3 different areas at 40x microscope objective. The scores were as follows; - (none): absent, 1+ (mild): a few immunopositive cells, 2+ (moderate): focal prominent immunopositivity and 3+ (intense): strong immunopositivity in more than 50% of the cells.

**RESULTS**

**C-ELISA Results and Immunohistochemistry Findings**

**C-ELISA Results:** C-ELISA results revealed that the percentage of infection in sheep more than in goats were in sheep (10) positive cases from a total number (17) examined cases (58.8%) on the other hand in goats (6) positive cases from a total number (15) examined cases (40%). Table 1 showing that there were a great similarity between C ELISA results and the intensity of PPR antigen persistence in different examined cases except in cases No. (55, 106) in sheep and cases No. (90) in goats. Tissue localization of immunolabeling indicating the viral presence; Also that was a complementary to histopathological findings: in addition to that Table (1) showing the intensity of antigen in intestine and lung more than in uterus.

**II-Case History and Clinical Signs:** The infected flocks suffered from sudden death in some cases and other cases showing severe profuse hemorrhagic watery diarrhea, acute respiratory distress characterized by shallow rapid breathing with discharges of clear strikes secretion from nose and mouth. In some chronic cases showing dry crusts around nose and muzzle. The main reproductive disorders we found are low conception rate and still birth. Abortion not detected in this study.

![Fig. 1: Showing severe pneumonia in most of lung tissue (yellow arrows) in comparison to apparently healthy tissue (blue arrow) accompanied with presence of hemorrhage soiled the ribs (white arrow) in ewes infected with PPR virus](image-url)
III-Histopathological Results

III-1-Macroscopical Findings: From our observation fibrinopurulent nasal discharge and crusts (especially in chronic cases) were present around the nostrils and on the muzzle, in other cases found ulceration and hemorrhage in the same areas. Lesions of the oral cavity showing ulceration in the dorsal and ventral surfaces of the tongue, in the lips and hard palates. Severe pneumonia and consolidation of different lobes of the lung and oozing of large amount of blood by taking a session in lung tissue. Heart showing hydropericarditis, the intestinal mucosa was severely congested and covered with mucous. Genital system apparently normal, generalized palness were evident in most cases of the liver.

III-2-Microscopical Finding: The main histopathological changes in this study is lymphocytic ulcerative inflammation accompanied with severe congestion in most of blood vessels and haemorrhages in female genital system and some visceral organs like small intestine; liver; heart.
Fig. 8: Intestine of PPR infected ewe showing necrosis of the whole intestinal villi and heavy lymphocytic infiltration in the inter glandular area of the basal area of intestinal villi. (black arrows) (H&E. X 100)

Fig. 9: Intestine of PPR infected ewe showing lymphocytic aggregation in between the intestinal glands; Also aggregation of the cynthetial giant cells (black arrow). (H&E. X 1000)

Fig. 10: Lung of PPR infected ewe showing peri vascular and peri bronchial infiltration of mononuclear inflammatory cells mainly lymphocytes and macrophages. (black arrow). (H&E X 400).

Fig. 11: Lung of PPR infected ewe showing intra alveolar oedema in most of pulmonary alveoli accompanied with diffuse infiltration of mononuclear inflammatory cells. (black arrow). (H&E. X 40)

Fig. 12: Liver of PPR infected ewe showing severe vacuolar degeneration in the cytoplasm of hepatocytes focal infiltration of lymphocytes. (black arrow). (H&E. X 400)

Female Genital System

Ovary: Ovary of non gravid ewes and does showing severe congestion of most of the blood vessels of C.T. stroma as in (Fig. 2) with degeneration and desquamation of the granulosa cells in some of mature follicles.

Oviduct: Showing folding and hyperplasia of the lining epithelium of the oviductal villi and desquamation of some of hyper plastic epithelial cells (Fig. 3).

Uterus: Uterus of non gravid ewes and does showing severe ulcerative lymphocytic endometritis characterized by partial desquamation of the lining epithelium of the endometrium accompanied with severe haemorrhages in most areas of endometrial stroma (Fig. 4). In some areas adjacent to ulceration found papillary hyperplasia (Fig. 5). Also found diffuse infiltration of mononuclear inflammatory cells mainly lymphocytes and macrophages in the submucosal layer and periglandular areas of the endometrium (Fig. 6). In the serosal layer found diffuse infiltration of lymphocytes (Fig. 7).
Fig. 13: Uterus of ewe showing multifocal golden brown positive immunoreactive staining of PPR antigen in infected inflammatory cells (black arrow) in the lamina propria submucosa of the completely ulcerated endometrium. ABC technique, Myer’s hematoxyline counter stain. (DAB x 200).

Fig. 14: Uterus of goat showing diffuse golden brown positive immunoreactive staining of PPR antigen in infected inflammatory cells (black arrows) in the lamina propria submucosa of the completely ulcerated endometrium ABC technique, Myer’s hematoxyline counter stain. (DAB x 200).

Fig. 15: Uterus of ewe showing diffuse golden brown positive immunoreactive staining of PPR antigen in infected inflammatory cells (black arrow) in the lamina propria submucosa of the completely ulcerated endometrium ABC technique, Myer’s hematoxyline counter stain. (DAB x 200).

Fig. 16: Lung of ewe showing diffuse golden brown positive immunoreactive staining of PPR antigen in infected inflammatory cells in the intra alveolar and interstitial tissue (black arrows) ABC technique, Myer’s hematoxyline counter stain. (DAB x 1000).

Fig. 17: Intestine of ewe showing golden brown positive immunoreactive staining of intracytoplasmic PPR antigen in the severely degenerated and desquamated enterocytes (black arrows) ABC technique, Myer’s hematoxyline counter stain. DAB x 200.

Fig. 18: Intestine of goat showing golden brown positive immunoreactive staining of intracytoplasmic PPR antigen in the severely degenerated and desquamated enterocytes (black arrows) ABC technique, Myer’s hematoxyline counter stain. DAB x 400.
Visceral Organs

**Small Intestine:** Necrosis in some areas along the whole length of the intestinal villi accompanied with diffuse lymphocytic infiltration around the intestinal glands other areas showing severe and diffuse infiltration of mononuclear inflammatory cells mainly lymphocytes and macrophages in the core of the intestinal villi (Fig. 8); Also giant cells neighboring to the infiltrated lymphocytes in the base of the intestinal villi (Fig. 9).

**Lung:** Severe lymphocytic interstitial pneumonia in most areas characterized by diffuse infiltration of peribronchial and peri vascular infiltration of mononuclear inflammatory cells mainly lymphocytes and macrophages (Fig. 10) accompanied with intra alveolar edematous fluid infiltration (Fig. 11).

**Liver:** Vacuolar degeneration along the most areas of liver tissue was found accompanied with periportal focal areas of lymphocytic infiltration in between hepatocytes (Fig. 12).

**Immunohistochemistry Results:** Moderate positive immunolabeling reaction was detected in endometrial mucosa, mostly in cytoplasm of the infiltrated macrophages which is multifocal distributed in the lamina propria submucosa of uterus (Fig. 13, 14 and 15). Additionally, in the interstitial pneumonic areas in infected lungs found the golden brown coloration of positive immunoreactive staining of PPR virus antigen in infiltrated macrophages (Fig. 16). The intestinal glands also showed strong positive immunoreactivity in the sloughed and degenerated enterocytes (Fig. 17 and 18).

**DISCUSSION**

Peste des Petits Ruminants (PPR) is an acute viral disease of goats and sheep characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis and pneumonia. [7]. PPR is one of the major notifiable diseases of the world organization for animal health (OIE) and existence of PPR can have a catastrophic impact on a region's farming development [20].

A possible role of camels in the dissemination of PPR virus to goats has also being suggested [21]. Peste des Petits Ruminants virus infection resembles rinderpest in terms of clinicopathological findings and pathogenesis. However, lung lesions resulting from PPRV infection differ from those resulting from rinderpest [11].

In this study we found that the susceptibility for PPR virus infection in sheep (58.8%) more than in goats (40%) this results explained by many reports said that; PPR virus is sometimes referred to as a more serious disease of goats than sheep, however, reports recorded sheep and goat populations have been equally reported [16, 22-24]. Also other reports added that; in some outbreaks goats appear not to be affected, while sheep succumb with high rates of mortality and morbidity [25].

According to reports which explain the pathogenesis of the disease, the virus enters through nasopharyngeal mucosa and localizes to and replicates in the regional lymph nodes [11, 14]. After a vireamic stage, virus can be identified in most lymphoid tissues, alimentary and respiratory mucosa and it subsequently causes pneumoenteritis [26]; that come in agreement with our finding from severe interstitial lymphocytic pneumonia and severe necrotic, hemorrhagic enteritis in most of examined cases as in (Fig. 8, 9, 10 and 11).

In the present study, characteristic ulcerative lymphocytic inflammation and necrotic lesions were found mainly in uterus, intestine, lungs and liver as in (Figs. 4-12); also immunopositive antigen which were detected in these organs previously reported specially inside the lining epithelium of the intestinal glands revealed the affinity of the PPR virus to the epithelial cells that come in accordance with Kul et al. [27]. Who reported that characteristic erosive ulcerative lesions that include syncytial cells and inclusions in the alimentary and respiratory mucosa and bronchointerstitial pneumonia reveal affinity of the PPR virus to the epithelial cells.

Peste des Petits Ruminants virus can cause severe lymphocytolysis in lymphoid tissues, such as tonsils, spleen, Peyer’s patches, mediastinal and mesenterial lymph nodes, subsequent immunodeficiency, to lymphoid depletion [13-15].
Infection of pregnant animals with the virus has also, albeit rarely, been linked to abortion. The importance of this and the mechanisms by which it occurs are currently unknown [9] although co-infection with both PPR virus and pestiviruses in cases of abortion has been reported [10].

The highly contagiousness and the easily transmitting by direct contact between the secretions and/or excretions of infected animals and nearby healthy animals as reported by Ezeibe et al. [4]; also our immunohistochemical finding in this study which confirm the ability of PPR virus to gain access inside the macrophages in uterus and lungs also in the intestinal gland cells can be explained by the antigenic structure of PPR virus were it has eight proteins including six structural proteins namely: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin protein (H) and the large polymerase protein (L) and two nonstructural proteins V and C [28]. As ‘herringbone-like’ structures of approximately 14-23 nm in length [8,29]. Three viral proteins: M, F and H are associated with the viral envelope which is derived from the host cell membrane. The M protein is located inside the envelope and serves as a link between the RNP and the two external viral proteins, F and H and is believed to be important for virus particle assembly. The virus binds to the host cell receptor through H during the first step of the infection process. Following attachment, F mediates the fusion of the viral envelope with the host cell membrane, introducing the viral RNP complex into the cell cytoplasm. F and in particular, H are considered to be very important for inducing protective host immune response against the virus [30].

CONCLUSIONS

PPR virus can be gain access to the reproductive system causing ulcerative lymphocytic endometritis; Also PPR virus antigen could be detected by immunohistochemistry in uterus so affect in the reproductive performance of sheep and goats.

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REFERENCES


