

STUDIES ON PESTE DES PETITS RUMINANTS (PPR) IN EGYPTIAN SHEEP

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SUMMARY

Three sheep flocks (at Bolack El-Dakror, El-Omrana El-Sharkia and El-Moneeb, Giza Governorate) were affected with rise of temperature, nasal and lachrymal discharges, coughing, erosions of the buccal mucosa and corneal opacity in three cases. The rate of infection was 0.11%, 0.3%, and 0.4% among the three flocks respectively. The course of the illness was about 10-12 days while, the outbreak period was 2 months.

At the peak of fever, two animals were slaughtered where the postmortem lesions were recorded. PPR virus was isolated on VERO cell from lungs, lymph nodes, liver and spleen. Virus neutralization test, AGPT, Solid and Dot ELISA confirmed that the isolate was PPR virus. It was as PPR-Giza 94. Histopathological examination revealed the pathological effects of the virus on lymph nodes, lungs, and liver. Inoculation of susceptible goats with the isolated virus showed signs similar to those on affected sheep. The virus was recovered from these experimentally infected animals.

INTRODUCTION

Peste des Petits ruminants (PPR) is an acute

contagious viral disease of goats and sheep, characterized by fever, oculonasal discharge, stomatitis, diarrhoea and pneumonia (Durojaiye and Taylor 1984). PPR is a morbillivirus infection of sheep and goats causing pyrexia, oral necrosis, diarrhea and death (El-Hag Ali and Taylor, 1984).

An outbreak of a disease with clinical signs suggestive of rinderpest occurred in South India among a flock of 800 sheep where 80 animals were found dead. (Shaila, et al., 1989). Splenic material was used for virological tests. After several passages in VERO cells and Hep-2-cells, the causative virus was isolated and finally identified as PPR.

The diagnosis of PPR infection became more important as it has been reported that PPR might not be restricted to West Africa as previously though (Majiyagbe et al., 1984). Currently, diagnostic procedures, used for PPR, include the agar gel precipitation test (AGPT) for detection of PPR antigen in lymph nodes and other tissues, animal inoculation, virus isolation and identification and serum neutralization test. The AGPT, SNT and virus isolation are the routinely used methods for PPR diagnosis (Majiyagbe et al., 1984).

Appel et al., (1981) mentioned that the postmortem findings, in PPR affected animals, were necrosis of the pharyngeal mucous

membrane and multiple linear erosions extending down the oesophagus. Area of congestion could be found in the abomasum, in the presence of normal mucosa of the small intestine. Payer's patches were prominent and oedematous. The capillaries of the mucosa, along the linear folds of the mucosa, along the linear folds of the caecum, colon and rectum, were congested (Zebra striping). Most affected animals with PPR showed pneumonic lesions with areas of red consolidation (Scott 1981). The Payer's patches in the small intestine might be grossly prominent with oedema (Appiah 1982).

It was found that PPR virus could be detected in the mesenteric lymph nodes of affected animals as well as the whole lungs (Appel et al., 1981). PPR viral antigen was detected in 33 out of 38 randomly selected caprine tissue homogenates using Dot-ELISA (Obi and Ojeh 1989). An outbreak of PPR disease appeared in January, 1987 among goats in a private farm at Giza Governorate, Egypt, where 21 animals (30%) died. (Ikram et al., 1988). PPR virus could be isolated on VERO cells from lymph nodes and spleen of two sacrificed goats. The virus antigen was detected in the mesenteric and bronchial lymph nodes using agar gel precipitation test. The virus was identified serologically using direct fluorescent antibody technique (FAT).

The gross lesions, seen in infected goats, included dehydration, soiling of the hindquarters with faeces, oedema and excoriation of the lips, discrete or extensive area of erosions, ulceration and necrosis on the various parts of oral cavity and nasal mucosa while the trachea in many instances contained mucopurulent or frothy exudate (Obi et al., 1983). In the majority of dead animals, pneumonic lesions were consolidation of the anterior lobes of the lungs. The spleen often appeared contracted and the liver was pale. The small intestine showed only occasional congestion. The large intestine showed congestion

of the capillaries, oedema of the linear folds of the spiral colon and rectum and congestion in the caeco-colic junction. The mesenteric lymph nodes were in general oedematous and congested. The pulmonary histopathological lesions were characterized by hyperplasia of the bronchial and bronchiolar epithelia, accumulations of dequamated epithelial cells, neutrophils and macrophages in the bronchiolar lumina, thickening of the alveolar walls and infiltration of the alveolar spaces by neutrophils, macrophages, plasma cells and giant cells. The splenic lesions consisted of congestion and lymphocytolysis while the liver showed multifocal areas of coagulative necrosis and vacuolation of the hepatocytes beside hyperaemia (Ikede 1983).

The objective of this work was to investigate 3 outbreaks of infectious disease among 3 herds of sheep in order to reach a diagnosis using the various tools of diagnosis, for isolation and identification of the causative agent, beside the associated lesions.

MATERIAL AND METHODS

I- Field outbreaks:

Three outbreaks were investigated at Bolak El-Omrana, El-Sharkia and El-Moneeb, Giza Governorate. These involved three herds of sheep. The three outbreaks occurred from May to July, 1994; History and clinical signs were recorded. Animals that were slaughtered during illness were necropsied and specimens of lungs, spleen, liver and lymph nodes were collected for histopathology and virological diagnosis.

2- PPR-hyperimmune serum:

It was prepared in goats according to Adu and Joannis (1985) by inoculating susceptible goats with a cell culture adapted PPR virus

(Egypt-87).

3- Rinderpest-hyperimmune serum:

Rabbit anti-rinderpest hyperimmune serum was supplied by Pirbright Surrey, England. It was used to differentiate between PPR and RP viruses using AGID and virus neutralization tests.

4- Peste des Petits ruminants virus (PPRV):

A VERO cell adapted PPRV (Egypt-87), obtained from Ames-Iowa Laboratories, U.S.A., was used for preparation of hyperimmune serum and serum neutralization test.

5- African Green Monkey cells (VERO):

VERO cell line established by Yasumura and Kawatika (1963) was used in this study for virus isolation and virus neutralization tests.

6- Cell Culture media:

Minimum Essential Medium (MEM) with Hanks salts (Eagle 1959) was used for cell culture passages. MEM was supplemented with newborn media and 2-3% for maintenance media.

7- Virus Isolation:

Samples of spleen, mesenteric lymph nodes, lungs and liver were collected from slaughtered animals where portions of each were ground and subjected to virus isolation procedures according to Furley et al., (1987) and Rossiter and Jessett (1982).

8- Applied serological tests:

8-1. Agar gel immuno diffusion test:

It was done using homogenates of lymph nodes, spleen, and lung of infected animals as antigen (Nawathe 1980).

8-2. Solid-Phase-ELISA:

It was carried out according to Anderson et al.(1982).

8-3. Dot ELISA:

It was carried out according to Hassan (1987).

8-4. Virus neutralization test:

It was carried out according to Rossiter and Jessette (1982).

9- Preparation of histopathological sections:

Specimens of lymph nodes, lungs, and spleen, from slaughtered animals, were sectioned at 5 micron thickness and stained with H&E according to Carleton (1967).

10- Animal Inoculation:

Three susceptible goats were inoculated intranasally with a homogenate of the collected samples and were kept separately under observation till the clinical signs appeared and then slaughtered.

RESULTS

The postmortem findings in slaughtered animals were mild pleuritis, pneumonia, congested bronchial lymph nodes, erosive buccal mucosa and haemorrhagic gastroenteritis.

The detection of viral antigen in the bronchial and mesenteric lymph nodes, lungs and spleen specimens from the two slaughtered sheep revealed positive reaction to PPR hyperimmune serum but not to RP one (Table 1).

PPR virus could be isolated on VERO cells from lymph nodes, lungs, liver and splenic specimens from both animals showing its characteristics cytopathic effect (Table 2). Such cytopathic effect started at the third day post inoculation beginning with cell rounding and advancing till reaching the

Table(1): Results of AGID test carried out with samples obtained from two slaughtered affected sheep.

| Animal Number | Samples | Hyperimmune serum | |
|---------------|------------------------|-------------------|-----|
| | | RP | PPR |
| 1 | Bronchial lymph nodes | - | + |
| | Mesenteric lymph nodes | - | + |
| | Lungs | - | + |
| | Liver | - | + |
| | Spleen | - | + |
| 2 | Bronchial lymph nodes | - | + |
| | Mesenteric lymph nodes | - | + |
| | Lungs | - | + |
| | Liver | - | + |
| | Spleen | - | + |

Table(2): Cytopathogenicity on VERO cells produced by isolate suspensions prepared from specimens obtained from two slaughtered affected sheep.

| Samples | Cytopathogenicity on VERO cells | |
|------------------------|---------------------------------|------------|
| | Sheep No.1 | Sheep No.2 |
| Bronnchial lymph nodes | ++++ | ++++ |
| Mesenteric lymph nodes | +++ | ++++ |
| Lungs | ++++ | ++ |
| Liver | ++++ | ++++ |
| Spleen | ++++ | ++++ |



Fig. 1- Lung showing thickened pleura. H&E. x300.

Table (3): Results of serological confirmatory tests done for identification of PPRV isolate.

| Test | Bronchial L.N. | | Mesenteric L.N. | | Lungs | | Spleen | |
|----------------------|----------------|-----|-----------------|-----|-------|-----|--------|-----|
| | RP | PPR | RP | PPR | RP | PPR | RP | PPR |
| Solid-phase ELISA | - | + | - | + | - | + | - | + |
| Dot-ELISA | - | + | - | + | - | + | - | + |
| Virus neutralization | - | + | - | + | - | + | - | + |

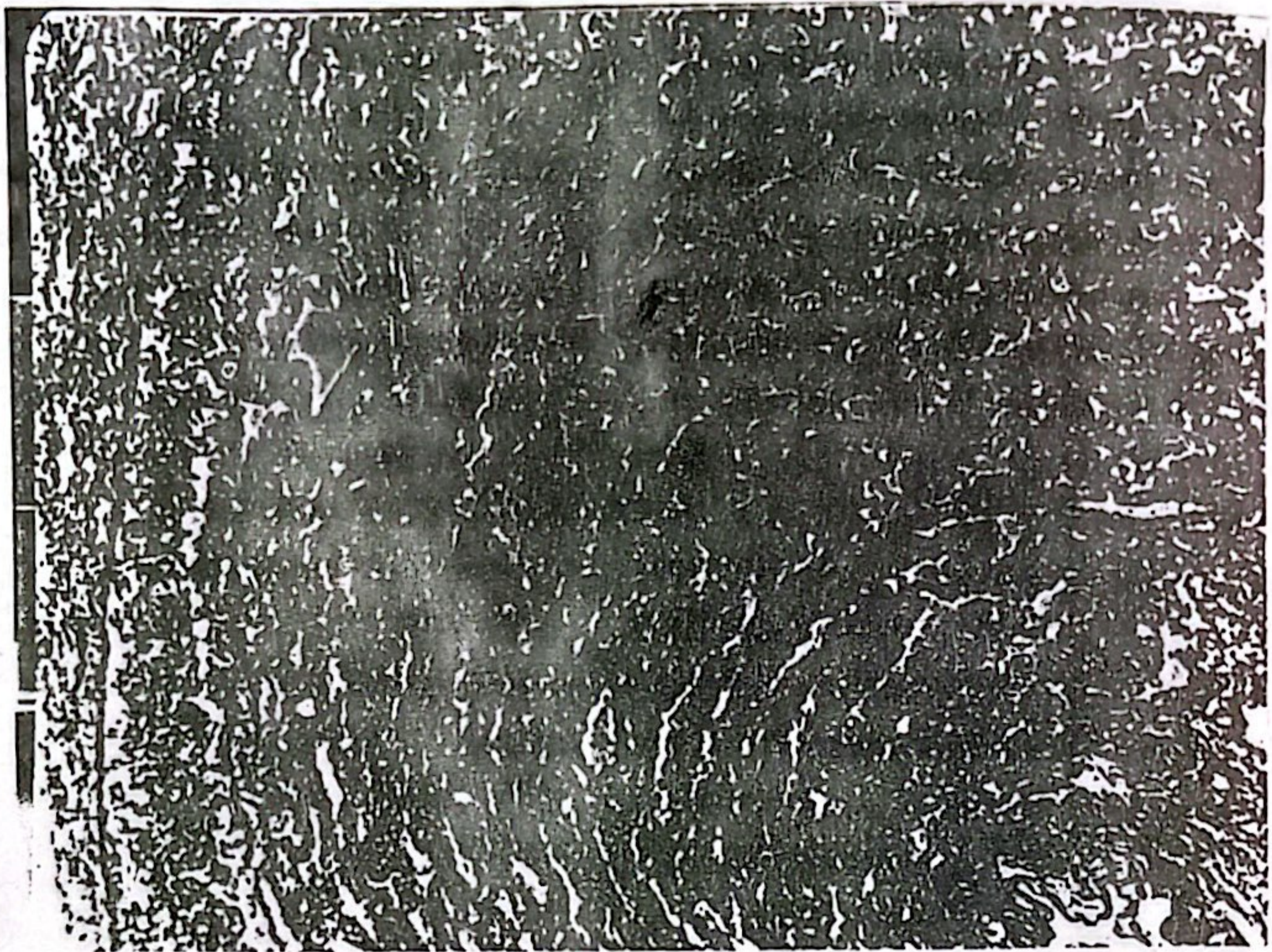


Fig. 2- Liver showing degenerative changes. H&E. x300.

stage of giant cell formation by the fifth day then complete cell degeneration with the picture of cell anastomosing processes. The isolate was confirmed to be PPR by solid-phase ELISA and virus neutralization tests.

The histopathological examination of lymph node revealed hyperplasia of the histiocytes which packed both the cortical and medullary zones. The lungs showed oedema and bronchitis with destroyed bronchiolar epithelium. The bronchiolar exudate contained mainly lymphocytes and plasma cells. Lungs consolidation was due to diffuse inflammatory cell infiltration and alveolar cell exudate. Pleuritis (Fig.1) and fibrinous pneumonia were detected in one animal. The liver showed diffuse hepatic degeneration (Fig.2) oedema and intensive hepatic focal inflammation. This findings were accompanied by minute

coagulative necrosis.

All the three inoculated goats showed both the clinical and postmortem findings resembling those recorded in the slaughtered sheep but in a mild form. PPR virus was recovered from the lymph nodes and spleen of the experimentally infected animals.

DISCUSSION

Peste des petits ruminants (PPR) is a contagious viral disease of small domestic ruminants characterized by pyrexia, catarrhal nasal and ocular discharge, necrotic stomatitis and an intestinal mucosal and lymphoid tissue reactional syndrome, in addition to the respiratory manifestation. The recorded observations, in

present study, agree with those reported by Appiah (1982), Durojaiye and Taylor (1984) and El-Hag Ali (1984).

The postmortem examination of slaughtered sick sheep and sacrificed experimentally infected goat revealed the pathognomonic lesions that have been reported by Appel et al., (1981), Appiah (1982) and Obi et al., (1983). Demonstration of PPR-virus antigen in the collected specimens by the AGID, confirmed the previously obtained results by Majiyagbe et al., (1984).

PPR virus could be successfully isolated on VERO cells from lymph nodes, lungs and spleen of both naturally infected sheep and experimentally infected goats and the isolated virus showed its characteristic cytopathogenicity. Similar results were previously obtained by Ikram et al., (1988) and Shaila et al., (1989).

The applied serological investigation (Solid-Phase-ELISA, Dot-ELISA and virus neutralization test), pointed out that the isolate was confirmed to be PPR virus. Such serological tests for identification of PPR virus have been previously used by Majiyagbe et al., (1984) and Olaleye et al., (1989).

The histopathological examination of sectioned specimens revealed pictures that were similar to those described by Appiah (1982). The reaction of goats to experimental infection with the isolated PPR virus were represented by specific clinical picture and postmortem lesions, almost similar to those reported by Bundza et al., (1987).

It could be concluded that PPR is among the important viral diseases that threaten the sheep and goats industry in Egypt. Protective measures against such disease should be considered.

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