ABSTRACT

It has been demonstrated that positive tissue samples from an active case in Turkana had viral RNA from a lineage III PPRV and the first available genome sequence was determined. Seven goats and sheep aged between 3-6 months that were tested to be negative for PPR antibodies by competitive-ELISA were used for study. These animals were divided into two treatment groups and one control group. Each animal in the treatment groups was inoculated through intranasal route with 2ml of 30% infected mixed tissue suspension while the control group was inoculated with phosphate buffered saline. Animals were then examined daily for development of PPR clinical signs. Clinical signs were observed in treatment groups while the control remained apparently healthy. Fever started to develop from 6.6±1.14 and 8.6±1.34 days post infection (dpi) in goats and sheep respectively. There was a progressive rise in respiratory rates from 9 to 16 dpi in goats and from 9 to 14 dpi in sheep. Nasal discharges were recorded from days 8.2±2.28 and 9±1.83 post infection in goats and sheep respectively. Ocular discharges were observed from days 10±2.24 and 9.8±2.17 post infection in goats and sheep respectively. Oral lesions were observed only in one goat and two sheep. Diarrhea was observed from day 13.5±0.58 post infection in sheep and from day 14±1 post infection in goats. Gross pathology revealed lesions mainly in the lungs, body lymph nodes and the intestines. The results from this study indicate that whereas PPR is thought to mainly affect goats, the disease in Kenya appear to evenly affect both, goat and sheep.
1 Introduction

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants, which is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis and ulceration of the mucous membrane and inflammation of the gastro-intestinal tract leading to severe diarrhea and high mortality (Gibbs et al., 1979). The disease was first described in Cote d’Voire in West Africa in 1942 (Gargadenne & Lallana, 1942). In Kenya, the disease was first detected in the Turkana County in 2006. After this it rapidly spread to 16 other districts such as West Pokot, North Pokot, Baringo, Samburu, Moyale, Marakwet, Marsabit, Wajir, East Pokot, Laikipia west and Injara and current reports indicates that the disease has spread to most of the country (Pro Med-Mail, 2008).

Natural disease affects both goats and sheep, but it is more severe in goats where it causes severe morbidity and mortality while occasionally it becomes severe in sheep (Raghavendra et al., 2000). However, a severe experimental form of disease has been reproduced in sheep and goats (Bundza et al., 1988). PPR virus has been mainly transmitted through close contact between infected and non-infected susceptible animals, which occurs in common grazing areas (Gargadenne & Lallana, 1942). The virus can be transmitted experimentally through different parenteral routes; intra-tracheal, subcutaneous injection of the virus (Couacy-Hymann et al., 2007), intranasally (Bundza et al., 1988) or by contact (Durojaiye, 1980). In Kenya, the description of clinical and pathological changes of lineage III PPR virus has not been documented in a controlled experiment. This has hampered the clinical diagnosis of this disease that has continued to be confused with pneumonic pasteurellosis and other pneumonic diseases of small ruminants. This study was aimed at understanding the clinical course and pathological changes in sheep and goats experimentally infected with Kenyan isolates of Lineage III Peste des petits ruminant virus.

2 Materials and Methods

2.1 Experimental animals

Seven goats and sheep aged between 3-6 months were tested and confirmed for the negativity for PPR antibodies using competitive ELISA. The animals were divided into three groups (A, B, C). Group A had five goats; group B had five sheep while group C had two sheep and two goats. Group A and B were the treatment groups while group C was the control. Groups A and B were housed in different rooms located within the same area while group C was housed in a separate area for bio-safety measures. The animals were allowed two weeks to acclimatize and were fed on hay and bran daily at 9.00am while water was provided ad libitum. Each animal was de-wormed with Valbazen™ at a dosage of 15mg/kg body weight.

2.2 The virus inoculums

The inoculum used in this experiment was prepared from tissues harvested from goats that were naturally infected with PPR in Turkana County, Kenya. Previously, it was well documented that the positive tissue samples had viral RNA from a lineage III PPRV and the first available complete genome sequence was determined (Dundon et al., 2014). Thirty percent of tissue suspension was prepared from a pool of homogenized lymph nodes, spleen, lungs and large intestines. Following centrifugation at 1500 rpm for 10 min, the supernatant was collected and antibiotics were added. The presence of PPR virus in the tissue suspensions was confirmed by real time reverse transcriptase polymerase chain reaction.

2.3 Baseline Study

All animals in the study were examined daily before inoculation for any signs of disease. Parameters including heart rate, respiratory rate and rectal temperature were monitored and findings recorded.

2.4 Animal Inoculation

Each animal in the treatment groups (group A and B) was inoculated with 2ml of 30% mixed tissue (lungs, spleen, large intestines and lymph nodes) suspension through the intranasal route. During infection, a small piece of swab was soaked with the suspensions and placed in the nasal cavity for about 5 minutes. The animal was held with the head facing upwards so as to avoid sneezing out of the swab while allowing it to take deep breaths. Each animal in the control group was administered with 2ml of phosphate buffered saline. All animals were then examined twice daily for the development of PPR signs as described by Roeder et al. (1994). This experimental infection of animals was approved by the Board of Postgraduate Studies, University of Nairobi, following the recommendations from the Faculty of Veterinary Medicine, having been satisfied that all bio-safety, animal use and ethical issues were addressed.

2.5 Gross and histopathological study

Sheep and goats that became moribund were sacrificed and a detailed post-mortem examination performed. The infected sheep and goats that did not succumb to infection were also sacrificed at the end of the study (20 dpi). Samples of lungs, spleen, lymph nodes and intestines were preserved in 10% neutral buffered formalin and processed for histopathology. Paraffin-embedded tissues were sectioned at 5μm, processed and stained with Haematoxylin and Eosin. Stained sections were examined under light microscope attached to a digital camera (x16.1 mp) and photographs were then taken. The histo-pathological lesions were ranked depending on the extent of the injury observed under high power field. Histopathological changes observed included: accumulation of exudates within the bronchioles, thickening of bronchiolar...
epithelium and alveolar atelectasis in the lung tissues; lymphoid cells depletion particularly in the cortex and medullary cords in lymph nodes; villous atrophy, infiltration with mononuclear cells into lamina propria and submucosal edema in the intestines. Injury observed was categorized as None (-), Mild (+), Moderate (+++) and Severe (++++) depending on the extent and distribution in the tissue.

3 Results

3.1 Clinical Response to in experimental animals

3.1.1 Fever

Before infection, the mean daily rectal temperature for goats and sheep was 38.65°C ±0.08 and 38.76°C ±0.03 respectively. Rectal temperature ≥39.5°C were considered high. Fever developed from 5 to 8dpi (mean 6.6 ±1.14) in goats and lasted for 5.6 ±1.95 days, before gradually subsiding. In sheep, fever developed from day 7 to 10 post infection (mean 8.6 ±1.34) and lasted for 6.2 ±2.49 days before eventually subsiding. The highest mean temperature for infected goats was 40.3°C ±0.45 recorded on 9dpi, while the highest mean temperature for infected sheep was 40.16°C ±0.22 recorded on day 12 post infection.

The highest individual rectal temperature recorded for both goats and sheep was 40.7°C on days 9 and 11 respectively. The mean daily rectal temperature in the treatment and control groups are illustrated in Figure 1 below. During this phase of disease all infected animals were generally dull, depressed and anorexic. The animals in the control group showed no thermal response.

![Figure 1 Mean daily rectal temperature in experimentally infected animals and controls.](image1)

![Figure 2 Daily mean respiratory rates in experimentally infected animals and controls.](image2)
3.1.2 Respiratory signs

In addition to the febrile response, respiratory changes were also clinically manifested in all infected animals. Prior to infection the mean daily respiratory rates in goats and sheep were 21.9±1.51 and 21.8±0.23 breaths per minutes respectively. However, following infection, there was a progressive rise in respiratory rates from day 9 to day 16 in goats (Figure 2), while the highest mean respiratory rate 34±4.47 breaths per minutes was recorded on 16 dpi. In sheep there was a progressive rise in respiratory rates from day 9 to day 14, while the maximum mean respiratory rate of 36±3.16 was recorded on 13 dpi. All infected animals developed moist rales either unilaterally or bilaterally by 10 to 14dpi. Thoracic ascultation revealed increased vesicular sounds that later became coarse crackles in the lower portion of the chest. Nasal discharges were recorded from 8.2±2.28 and 9±1.83 dpi in goats and sheep respectively. These discharges were initially clear and watery but later became whitish and mucoid as the disease progressed and predisposed the animals to mild dyspnea. Coughing was observed in two goats (goats 1 and 2) on 14 and 15dpi.

3.1.3 Ocular Discharges

In goats, ocular discharges were observed on day 10±2.24 post infection. These discharges were clear and watery except in one goat where they become thick and yellowish from 13 dpi. In sheep, the discharges were observed from day 9.8±2.17 post infection. The discharges were also clear and watery with exception of two sheep where they became whitish and mucoid on days 11 and 14 respectively. These discharges were initially scanty but increased in volume to about 4 ml as the disease progressed. These discharges resulted in the matting of the hairs below the eye. The conjunctiva of infected animals was reddened and the cornea of sheep 3 became cloudy [plot 1].

3.1.4 Oral lesions and diarrhoea

Among the tested animals (groups A and B), only one goat (20%) and two sheep (40%) developed oral lesions. These lesions were first observed on 9 dpi in one sheep, seen as a small nodular swelling on the skin on the outside of the lip around the commissure of the mouth. The swelling was firm and painless but later became filled with pus and painful to touch. Similar swellings developed in one other sheep and goat on dpi 12 and 14 respectively. These swellings would later on burst open oozing yellowish pus that formed scab. By day 17 post infection, the swellings had resolved in sheep forming a scar tissue. Four infected sheep (80%) developed diarrhea from day 13.5±0.58 post infection. It started as soft and pasty feces which later developed into severe foul smelling watery diarrhea with mucoid deposits. The diarrhea persisted for 3.75±0.957 days resulting in severe dehydration characterized by sunken eyeballs and loose skin. These animals became moribund and death followed on day 17±1.414 post infection. In goats, severe diarrhea was observed in three animals (60%) from day 14±1 post infection. These goats were euthanized on days 17, 18 and 19 respectively after they became moribund. The rest of the animals were euthanized and necropsied on day 20 post infection after clinical signs of PPR had gradually subsided. The occurrence and frequency of clinical signs have been summarized in Tables 1 and 2 below.

![Plot 1](image-url) An experimentally infected sheep was showing catarrhal ocular discharges on 14 dpi (white arrow). The conjunctiva was also reddened.

### Table 1 Days of the onset of clinical signs in experimentally infected goats and sheep (n=5).

<table>
<thead>
<tr>
<th>Species</th>
<th>Median incubation period</th>
<th>Fever</th>
<th>Ocular discharges</th>
<th>Nasal discharges</th>
<th>Oral lesions</th>
<th>Diarrhea</th>
<th>Day euthanized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goats</td>
<td>6 days</td>
<td>6.6±1.14</td>
<td>10±2.24</td>
<td>8±2.28</td>
<td>14</td>
<td>14±1</td>
<td>17-20</td>
</tr>
<tr>
<td>Sheep</td>
<td>7 days</td>
<td>8.6±1.34</td>
<td>9.8±2.17</td>
<td>9±1.83</td>
<td>10.5±2.12</td>
<td>13.05±0.58</td>
<td>17±1.414</td>
</tr>
</tbody>
</table>

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http://www.jebas.org
Table 2 Frequency of clinical signs in sheep and goat experimentally infected with PPRV mixed tissue suspension.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Fever</th>
<th>Nasal discharges</th>
<th>Coughing</th>
<th>Ocular discharges</th>
<th>Oral lesions</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goats</td>
<td>5/5</td>
<td>5/5</td>
<td>2/5</td>
<td>5/5</td>
<td>1/5</td>
<td>3/5</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>100</td>
<td>40</td>
<td>100</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Sheep</td>
<td>5/5</td>
<td>5/5</td>
<td>0/5</td>
<td>5/5</td>
<td>2/5</td>
<td>4/5</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

3.1.5 Post mortem findings

Generally, the carcasses were in poor body condition, severely dehydrated with sunken eyeballs and loose skin. The cornea was cloudy starting from the medial canthus extending towards the lateral of the eye. The hair around the perineum and hind limbs were matted due to diarrhea. The eyes and nose contained dried up discharges. The most characteristic lesions were found in the lymphoid organs, gastrointestinal tract and respiratory system.

Figure 3 Lung from an experimentally infected goat that was euthanized at day 20 post infection. Left diaphragmatic lobe appearing congested.

3.1.6 Respiratory system

The serosal and mucosal surfaces of the trachea were moderately reddened with streaks of congestion along the tracheal rings and the lumen contained mucoid exudates. The mediastinal lymph nodes were swollen and edematous. Lesions in the lungs were mainly confined to the diaphragmatic lobes and consisted of consolidation, diffuse reddening and atelectasis (Figure 3). The cut surface of the parenchyma oozed blood while the bronchioles contained mucoid exudates. One sheep (10%) had a sharply demarcated area on the left diaphragmatic lobe that was triangular shaped and oozed fluid with froth on cutting.

3.1.7 Lymphoid organs

Body lymph nodes, especially mesenteric lymph nodes were enlarged and edematous in all experimentally infected animals (100%). Their prominence presented a goose berry like appearance of the nodes stretching the mesentery (Figure 4). The mesenteric blood vessels were prominent and heavily congested. The mediastinal and pre-scapular lymph nodes were also enlarged and edematous. The spleen was slightly enlarged with rounded edges.

Figure 4 Swollen and enlarged mesenteric lymph nodes from an experimentally infected sheep having a goose berry-like appearance. The sheep died on 17 days post infection.

3.1.8 Gastro-intestinal tract

The esophagus was diffusely congested. The rumen, reticulum, omasum and abomasum were filled with semi-solid, thick, porridge-like blood tinged fetid smelling ingesta. The serosal surface of the intestine was diffusely congested with injected mesenteric blood vessels. The mucosal surface of the intestines was also diffusely congested with mucoid exudates in the entire length of the intestines. The liver was diffusely reddened and enlarged with rounded edges. The gall bladder was distended and filled with free flowing bile.

3.2 Histopathology: A summary of histopathological lesions is shown in Table 3

3.2.1 Gastrointestinal tract

In sheep, intestinal lamina propria revealed moderate infiltration with mononuclear cells including plasma cells and lymphocytes. Goblet cells filled with mucin were also prominent. Intestinal villi were severely reduced in height and blunt (Figure 5) while intestinal crypts were mildly filled with necrotic cells debris.
Moderate submucosal edema was also a prominent feature. In goats that expressed severe diarrhea, there was moderate infiltration with mononuclear cells with proliferation of goblet cells. Intestinal villi were moderately reduced in height with the intestinal crypts appearing clear. The sub mucosal edema was mild.

Figure 5 Showing severe atrophy of intestinal villi in a goat experimentally infected with PPRV (black arrow). (H&E X400).

3.2.2 Lymphoid organs

Body lymph nodes, especially the sub-mandibular and mesenteric lymph nodes revealed a variable degree of lymphoid cells depletion particularly in the cortex and in medullary cords. In sheep, there was moderate to severe lymphocytic depopulation with depopulated areas appearing pale, devoid of lymphoid follicles and with few or no lymphocytes (Figure 6).

Lymphoid depopulation left marked necrotic debris and prominence of supportive trabeculae network in the lymph nodes (Figure 7). Due to this depopulation, there was sharp loss of demarcation between cortex and medulla. The depopulation was due to severe lymphocytic necrosis. These lesions were more severe in mesenteric lymph nodes compared to other body lymph nodes. Mild blood vessels engorgement and edema were seen in medullary region. In goats lymphoid cell depletion was mild. Lymphoid follicles were observed within the outer cortex. The germinal centers of these follicles were pale and devoid of mature lymphocytes with intact and mature lymphocytes being observed within the inner cortex. The medullary sinuses and chords were mildly depleted of lymphocytes. Lesions in the spleen were less severe compared to those in the lymph nodes.

3.2.3 Respiratory system

The histopathological lesions observed within the respiratory tract were few, mild and often within the lung tissue. These lesions were characterized by accumulation of exudates within the bronchioles (Figure 8) and thickening of bronchiolar epithelium. Three sheep had moderate alveolar atelectasis with alveolar openings having a slit like appearance. Three goats had mild alveolar atelectasis and the other two had thickened inter-alveolar septae due to increased histiocyte proliferation.

Table 3 A summary of histopathological findings in goats and sheep experimentally infected with peste des petits ruminant (PPR) virus tissue suspension.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Lungs</th>
<th>Gastrointestinal tract</th>
<th>Lymphoid organs (depletion and necrosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alveolar atelectasis</td>
<td>Thickening of inter alveolar septae</td>
<td>Thickening of bronchiolar epithelium</td>
</tr>
<tr>
<td>Goat 1</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Goat 2</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Goat 3</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Goat 4</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Goat 5</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sheep</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sheep</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sheep</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sheep</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: - None, + Mild, ++ Moderate and +++ Severe.
4 Discussions

4.1 Clinical signs

The experimental infection of goats and sheep with PPRV tissues suspension revealed clinical signs such as fever, ocular-nasal discharges, diarrhea and dehydration and post mortem lesions in infected animals. The disease appeared to be subacute with its incubation period in goats and sheep being 6.6±1.14 and 8.84±1.30 days respectively.

Fever appeared to be moderate with highest rectal temperature recorded being 40.3±0.45 and 40.16±0.22°C for both goats and sheep respectively. This is in agreement with Diallo (2006) who described the subacute form of PPR infections as having longer incubation period of up to 6 days with affected animals recording low grade pyrexia of 39-40°C. In this study, nasal discharges were recorded in all infected animals and although they changed from clear watery to whitish mucoid discharges, they only caused moderate dyspnea. This explains why there was a rise in respiratory rate in infected animals This is in contrast to acute form of disease where severe signs of pneumonia such as noisy respiration with extended head and neck, nostrils dilatation, gasping with protruded tongue and painful coughs have been reported (Bundza et al., 1988).

Ocular discharges and oral lesions also appeared to be mild with the latter being observed in only three animals.

Oral lesions appeared in form of small papules at the commissure of the mouth that burst open forming scabs. This is in contrast to acute form of PPR where severe oral lesions characterized by rough necrosis begin on the lower gum and covers the dental pad, hard palate, inner side of the cheek, dorsal part of the tongue and around the commissures of the mouth (Abubakar et al., 2008). Areas where these papules form have been confirmed to be the predilection sites for PPR virus replication and thus could indicate tropism towards the skin of the lips (Al Naeem & Abu-Elzein, 2008). Generally, from this study, clinical signs appeared to be mild except for diarrhea which became severe with time. This is in agreement with Mann et al. (1974) who suggested that successful transmission of acute disease may require more than one challenge. El Hag (1973) also found that animals experimentally infected with PPRV develop mild clinical form of disease. In this study, goats and sheep appeared to be evenly affected with both species of animals exhibiting sub acute form of the disease. This was evident in the clinical signs observed whereby all infected sheep and goats recorded fever, nasal discharges and ocular discharges; this is contrary to the study of Raghavendra et al. (2000) who found that goats are more severely affected by PPR infections than sheep. This was however in agreement with reports in Asia and Middle East where both goats and sheep are evenly affected and PPR virus causes high mortality in both species of animals (Shaila et al., 1989). The PPR virus causing disease in Middle East and Asia has been identified to be of same lineage as the one causing disease in East Africa (Dundon et al., 2014) and this could explain why the experimentally infected animals appeared to be evenly affected. PPRV is both lymphotropic and epitheliotropic and therefore localizes in the lymphoid tissues and epithelium of gastrointestinal tract of infected animals. This therefore explains the increased incidence of oral lesions and diarrhea in the infected animals.
4.2 Necropsy

Necropsy findings reported in this study with rare exception were almost similar to those previously described by Sergany et al. (1992), Islam et al. (2001) and Pawaya et al. (2004) though the lesions appeared to be less severe. This exception was noted in the respiratory system where consolidation, diffuse reddening and atelectasis were observed in diaphragmatic lobes of the lungs contrary to earlier report where similar lesions were reported in cranial and apical lobes. The difference in severity of the post mortem changes between experimentally infected animals in this study and those reported in natural infection could be due to less load of virus used for infection, poor defense mechanism of naturally infected animals or secondary bacterial infection under field conditions (Osman et al., 2009).

Like other morbilli viruses, PPR virus is both lymphotropic and epitheliotropic and thus the pathological lesions are likely to be severe in organs rich in lymphoid and epithelial tissue (Scott, 1981). The PPR virus, after invading the host through the respiratory system mainly localizes in the regional lymph nodes, especially sub mandibular and tonsils, resulting in lymphopenia. This explains the observed moderate to severe lymphocytic depletion mesenteric lymph nodes mainly in sheep and moderate in goats. The lymphatics were distended while blood vessels were congested and this could explain why the lymph nodes were enlarged. The epitheliotropic nature of PPR virus could explain why there was moderate to severe loss of intestinal villi. Presence of goblet cells hyperplasia within the intestinal epithelium explains the mucoid diarrhea and the mucoid exudates that were observed on the intestinal mucosa at post mortem. Increased vesicular sounds that later became coarse crackles could have resulted from accumulation of exudates within the bronchi and bronchioles that were seen at the histopathological examination. Histopathological changes describes in this investigation correspond with the finding by others (Bundza et al., 1988; Dhand et al., 2003; Diallo, 2006).

Conclusion

This study shows that experimental infection of sheep and goats using lineage III PPRV from field cases in Kenya produced a disease that appeared to evenly affect both sheep and goats. The pertinent clinical signs have been traced to the respiratory, gastrointestinal, and lymphoid systems. Fever and ocular discharges are consistent in clinical disease. The course of infection appeared to be sub-acute compared to acute or peracute course that is reported in naturally infected animals. Therefore both clinical signs and post mortem lesions can be easily used for PPR diagnosis.

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