Sero-Prevalence of Peste Des Petits Ruminants Among Goats of Different Zones of District Thatta, Sindh

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Abstract: Introduction: Peste des Petits Ruminants (PPR) is a highly contagious and viral disease primarily affecting goats and sheep, caused by the PPR virus (PPRV), which belongs to the family paramyxoviridae, and genus morbillivirus. Methodology: A total of 100 blood samples (female n=67 and male n=33) of goats from different areas of Thatta district were collected. Risk factors like area, age, sex and season were analyzed. Clinically affected animals exhibited high temperature (41\(\degree\)C), anorexia, dullness, lacrimal secretions, and nasal discharge diarrhea starting from 2 to 6 days post infection, hair blow the eyes becomes wet and there is matting together of the eyelids as well as partial blockage of the nostrils by dried up purulent discharges. Samples were transported to Central Veterinary Diagnostic Laboratory (CVDL) Tandojam, Sindh for laboratory confirmation. The competitive ELISA was performed to measure antibodies to the PPR virus. Results: The sero-positivity of PPR cases in female were 76.12% (51/67) and male 51.52% (17/33). The highest sero-prevalence was observed in age group of 4-12 month 75.56% (34/45). Lowest sero-prevalence was detected in age group of 0-4 month 47.83% (11/23). Highest prevalence of PPR infections were observed in the month of August 70% (21/30), followed by 69.04% (29/42) in the September, the lowest prevalence was 64.28% (18/28) in the July. Conclusion: It is concluded from the present study that female animals were more affected than males. The higher infection was recorded during the August. Comparatively, young animals were more affected than the suckler and adults.

Keywords: Peste des petits ruminants, Seroprevalence, C-ELISA, Goat, Thatta, Sindh

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Introduction
Peste des petits ruminants (PPR), known as Goat plague locally called Kata. It is an infectious disease of sheep and goat, characterized by oculus, stomatitis, diarrhea and pneumonia [1]. PPRV was first time identified in the Ivory Coast in 1942 [2]. The disease is characterized by a range of clinical signs, including pyrexia, lacrimation, serous nasal discharge, anorexia, diarrhea, and pneumonia, with increased morbidity and mortality rates [3]. PPR initially results in a premature dullness of infected animals with high fever and anorexia, one to two days later, genital, ocular
and nasal mucosa inflammation leading to mucopurulent bronchopneumonia [4]. The Peste des petits ruminants virus (PPRV) transmission takes place from infected goats to cattle [5, 6] and its antigen has been detected in camels [7]. This virus is highly contagious, and it is transmitted directly through contact, excretions, or secretions of ill animals [8]. The Peste des petits ruminant’s virus was recognized as epidemic and endemic in Punjab, Pakistan [9]. This virus can circulate within small ruminants. Sub clinically, detection may be complicated, this characteristic of PPRV disease makes serological consideration problematic, especially in areas where caprine populations have been vaccinated as the serological isolation is not possible between vaccinated and obviously contaminated animals [10]. PPRV diagnosis is based on case history, location, clinical inspection and histopathological findings. However, conventional RT-PCR (reverse transcription polymerase to chain reaction) and Elisa (Enzyme linked immunosorbent assay) are routinely used for virus isolation [11]. The PPR disease has no effective treatment, but some antibiotics are used to stop secondary bacterial infections [12]. The PPR, infections have economic importance, and it poses a significant control towards successful small ruminant production because of high morbidity and death ratio [13]. The seroprevalence of PPR has been investigated in various regions, and studies have highlighted the presence of PPRV-specific antibodies in both goats and sheep [14]. India recorded 33.8% infections in goats and sheep. In their study, 504 samples were collected from goats and sheep showing signs of pneumo-enteritis. Seventy-nine samples were found positive for PPR with sero-prevalence of 15.6% when screened by RT-PCR. The transmission of PPR occurs through direct contact among affected and susceptible populations. The sero-prevalence of PPR was reported in caprine adult (>1 year), young (Between 4 to 12 months) and suckling kids and lambs (between 1 to 3 months) and found 10.15%, 31.06%, and 13.14%, respectively. The goats of less than one year of age were more susceptible to disease. Moreover, young goats need extra nutritional supply for their reproductive maturity and weight gain. It has been documented that the PPR disease was greater (28.52%) in male goats than nanny goats (13.04%). Males were more prone to the infection than females may be because of genetic factors [15]. The seasonal variation is practically responsible for the PPR prevalence in goats. Higher sero-prevalence of the disease was noticed in Rajshahi goats breeds during the months of December [16]. PPR virus enters through respiratory tract mucosa [17]. The virus reaches to regional lymphoid tissues to the replicates and spreads throughout the body via both the vascular and lymphatic systems. PPR virus can enter through occulo-nasal secretions [18]. Several studies have reported severe necrosis in lymphoid organs (thymus, pulmonary lymph nodes, spleen) and nearly 25% decrease in circulating peripheral blood leucocytes (leucopenia) caused by PPR infection [19]. Once the pathogen getting entry, affected animals likely shows acute pulmonary congestion and edema that causes death due to weaken immune system within a week [18]. Some animals may be able to survive for a month or more. The severity of infection depends on the capability of animal’s specific immune response against PPRV infection, parasitic and dietary state of the animal are some factors associated with co-infection which may lead to difficulty in differential diagnosis [20]. Therefore, this study aims to measure the seroprevalence and risk factors associated with PPR in goats of District Thatta.

Materials and Methods
Research work as carried out at Central Veterinary Diagnostic Laboratory, Tandojam, Sindh and Department of Veterinary Microbiology, Sindh Agriculture University Tandojam.

Sample And Sample Size
A total of 100 blood samples were collected from goats of district Thatta. Infected or non-infected animals divided according to the age-wise, for example 0-4 months old (Suckler), 04-12 months old (Young), and more than 12 months old as adults. Same as above strategy, they were divided month wise into three months categories (July, August, September). The groups were further sex-
wise divided into male and female. Five (5) ml blood samples were obtained from goats’ jugular vein using blood vacutainers. Collected samples were immediately stored in ice box and transported to the Central Veterinary Diagnostic Laboratory of Tandojam. Sera were separated at 10,000 rpm for 5 minutes and kept at -20°C before serological test. Sera Samples were processed by Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) for the confirmation of the positive samples.

**Data collection during sampling**
The history of the suspected animals using standard proforma consist of parameters such as sex, age, area, and month of collection.

**Recording of sign and symptoms**
Different signs and symptoms such as mucosal erosion, respiratory stress, secretion from mouth, nose, and eye, coarse coat and dirty hindquarters were observed. Temperature was recorded by indirect palpation per rectum using thermometer of every case and tabulated. Indirect auscultation was performed to hear the lung and tracheal sound to coincide with the symptoms of pneumonia.

**Competitive Enzyme Linked Immunosorbent Assay (c-ELISA)**
Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) were performed according to the diagnostic kit Vet Innovative Diagnostics (ID. Vet,310, rue Louis Pasteur-Grabels- France). The samples and the control were added in micro-wells. Briefly, 25 micro liter dilution buffers were used for dilution of the antibodies of PPR (Peste des petites ruminants) in each positive and negative control wells of the plate. Then test samples were incubated at 37°C (± 3 °C) for 45±4 minutes and washing of each well three times with approximately 300ml wash solution. One hundred (100) micro liters of conjugate were added into each well and incubated at 21°C for 30 minutes. Then three times washing was done using wash solution. In each well 100 micro liters of substratum solution was applied. Then each well was utilized with the stop solution to stop the reaction and visual density at 450nm. The percentage inhibition values of samples less than or equal to 50% were considered positive and negative for PPR virus antibodies were more than or equal to 60%.

**Results and discussions**
Peste des petits ruminants or kata”sero-prevalence was carried out in goats. During this research, 100 serum samples were tested, 25 serum samples each from Jhampir, Jhirk, Gharo and Mirpursakro areas of district Thatta of Sindh. The c-ELISA was performed to measure the activity of bound enzymes/antibodies in seroprevalence of PPRV.

**Sero-prevalence of PPRV district of Thatta**
**Area wise Sero-prevalence**
The results regarding serological prevalence of PPRV in caprine are presented in Table-1. The serological occurrence of PPR in goats was recorded as 84%, 72%, 64% and 52% in the areas of Jhampir, Jhirk, Gharo and Mirpursakro respectively. During present study highest sero-prevalence of PPR was detected in caprine of Jhampir area of the district Thatta.

**Table 1:** Area wise sero-prevalence of Peste des petits ruminants in goats.

<table>
<thead>
<tr>
<th>District Thatta</th>
<th>No. of Samples</th>
<th>No. of Samples positive</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jhampir</td>
<td>25</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td>Jhirk</td>
<td>25</td>
<td>18</td>
<td>72</td>
</tr>
<tr>
<td>Gharo</td>
<td>25</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Mirpursakro</td>
<td>25</td>
<td>13</td>
<td>52</td>
</tr>
</tbody>
</table>

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Gender wise sero-prevalence

The c-ELISA was performed in both male and female goats to estimate the sero-occurrence of PPR in caprine. PPR was observed as 76.12% in female goats and 51.52% in male goats (Table-2). Comparatively female goats were more infected than male.

Table 2: Sex wise sero-prevalence of Peste des petits ruminants in goats

<table>
<thead>
<tr>
<th>District</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>Samples</td>
<td>Positive samples</td>
<td>%</td>
<td>Samples</td>
</tr>
<tr>
<td>Jhampir</td>
<td>20</td>
<td>18</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>Jhirk</td>
<td>18</td>
<td>14</td>
<td>77.77</td>
<td>7</td>
</tr>
<tr>
<td>Gharo</td>
<td>13</td>
<td>9</td>
<td>69.23</td>
<td>12</td>
</tr>
<tr>
<td>Mirpursakro</td>
<td>16</td>
<td>10</td>
<td>62.5</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>51</td>
<td>76.12</td>
<td>33</td>
</tr>
</tbody>
</table>

Age wise Sero-prevalence

Highest sero-prevalence (75.56%) of PPR was observed in 04–12-month age group, whereas, group >12 month exhibited (71.88%). The lowest sero-prevalence (47.83%) was seen in age group of 0-4 months (Table-3).

Table 3: Age wise Sero-prevalence of Peste des petits ruminants in goats

| Area of Thatta | 0-4 month | | 4-12 month | | >12 month |
|----------------|-----------|---|-------------|---|----------|---|
|                | Samples | Positive samples | % | samples | Positive samples | % | Samples | Positive samples | % |
| Jhampir        | 5       | 4             | 80 | 12      | 10            | 83.33 | 8       | 7              | 87.5 |
| Jhirk          | 6       | 3             | 50 | 9       | 7             | 77.77 | 10      | 8              | 80 |
| Gharo          | 4       | 1             | 25 | 15      | 12            | 80   | 6       | 3              | 50 |
| Mirpursakro    | 8       | 3             | 37.5| 9      | 5             | 55.56 | 8       | 5              | 62.5 |
| Total          | 23      | 11            | 47.83| 45     | 34            | 75.56 | 32      | 23             | 71.88 |

Month (July, August And September) Wise Sero-Prevalence

The serum samples of both sexes (male and female) goats were analyzed using c-ELISA to record the sero-prevalence of PPR (Peste des petites ruminants) in goats. Highest sero-prevalence of PPR was observed in August (70%) whereas, lowest (64.28%) seroprevalence was observed July. Overall infection rate was higher in August as compared to July (Table-4).

Table 4: Seasonal sero-prevalence of Peste des petits ruminants in goats

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of samples</th>
<th>No. of samples positive</th>
<th>Positive sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>28</td>
<td>18</td>
<td>64.28</td>
</tr>
<tr>
<td>August</td>
<td>30</td>
<td>21</td>
<td>70.00</td>
</tr>
<tr>
<td>September</td>
<td>42</td>
<td>29</td>
<td>69.04</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>68</td>
<td>68</td>
</tr>
</tbody>
</table>

Sero-prevalence of Pest des petits ruminants were studied in goats of district Thatta. During present study 25 serum samples were collected and screened by c-ELISA from Jhampir, Jhirk, Gharo and Mirpursakro area of Thatta and showed sero-prevalence of 84%, 72%, 64% and 52% respectively. Higher sero-prevalence of 84% was observed in the Jhampir area of the district.
Thatta. The results of the present study were found in accordance with the findings published by [21] who reported 69.3 % sero-prevalence in hilly area of Dadu district Sindh, Pakistan. While higher sero-prevalence of 76% was recorded in Chakwal, followed by 75.42%, 64.29 %, 64.72%, 61.28%, 60.67% and 60.32% in districts Bahawalpur, Northern areas, Sahiwal, Azad Jammu and Kashmir (AJK) and Rawalpindi, Pakistan. However, the higher sero-prevalence of infection in Sargodha region may be due to epidemiological factors [22]. During the rainy season, the availability of nutrients for livestock improves due to increased fodder production, which may lead to increased resistance to disease as animals are restricted for grazing during rainy season. Moreover, young goats need further nutritional supplement for their body weight and sexual maturity. If they suffer from long term malnutrition it will result in the weakened immune system [22-23]. Deprived nutrition in the desert region will increase the incidence of disease due to a reduction in immune resistance by triggering immune system and this virus is lymphotropic in nature might have affected less immune animals in barren region of Thatta district. There was high sero-prevalence reported in irrigated plain of Thatta may be because of animal markets and small ruminants’ migration from desert to irrigated section under drought condition, moving easily and grazing on open pasture where infected animals’ spreading PPR virus in other non-infected animals. Nearly 70- 80% of the population of caprine is at the risk of PPR virus infection due to low doses of the vaccine. The climatic condition of this mentioned district, however, does not vary in great deal but strength has minor variation which could have altered the incident of disease [24]. Entry of fresh animals into flocks, which are mostly under stress during the traveling one area to another area and large distances and available of low nutritional during their migration, animals are mostly at risk during migration. This may be the main cause of the increase in high incidence of PPR occurrence due to low nutritional status [23]. In agreement with previous study [25], It was found the sero-prevalence of PPR is significantly higher in the females (76.12%) than males (51.52%). Moreover, [26] scientists reported on the bases of sex prevalence of antibodies corresponding to PPR, the occurrence was found to be higher in female (69.96%) as compared to male (63.92%). This may be related to the physiological differences between female and male, where females reveal some degree of infection resulting from stress due to milk production and pregnancies. Due to significance of productivity potential, females maintained for a longer period as compared to males, thus increasing the likelihood for female animals to be exposed to PPRV over time. In short, it is without a doubt concluded from the discussion the females are at high risk of PPR virus then the males. The highest recorded sero-positivity was 100% in goat in districts of Cholistan [22]. Sero-prevalence of PPR virus antibodies varies with three age groups under study. High sero-prevalence 34 (75.56%) was found in goat in age group 4-12 month whereas, lowest sero-prevalence 11 (47.83%) was in age group 0-4 month and lower sero-prevalence 23 (71.88%) was seen in age group >12 month. Resemblance findings of serological occurrence of anti-PPRV antibodies were detected more in adults (69.13%) than in sucklers 62.47% [27]. Movement of animals is one of main factor that causes outbreak of PPR in goat populations. Animals mostly travelling in dry season for long distance for the search out of water and fodder. Moreover, young animals need extra dietary supplement for their body weight gain. As a result, they suffered from long term malnutrition resulting in the loss of the immune system [22-23]. The age groups and sex and extrinsic factors suggested earlier outbreaks of PPR virus in endemic regions due to persistence of the virus [27].

In the current study higher occurrence of PPR infection was observed in the month of August (70%) and lower in month of September (69.04%) and lowest was detected in month of July (64.18%). Main cause of infection in month of August was due to dirty wind then rain, high humidity and moving freely on open pasture where infected animals spreading PPRV to non-infected animals. Most outbreaks in humid conditions can be attributed to survival of virus at low temperatures. Consistent with our findings previous studies have shown seasonal prevalence of
PPR in goats, the disease observed higher during the months of December (31.68%) and January (30.34%); and lowest in the months of June (9.52%) and July (11.86%).

Conclusions
PPR was detected with the highest sero-prevalence in goats of the Jhampir area in comparison to Jhirk, Gharo and Mirpursakro areas of district Thatta. Female goats were more infected with PPR than males. Young goats of 4-12 months showed highest sero-prevalence in comparison to sucklers and adults, however sucklers were less infected than young and adults. Higher sero-prevalence was found in the month of August as compared to July and September, and lower infection rate in July.

HUMAN AND ANIMAL RIGHTS
No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
None.

FUNDING
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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