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EVALUATING THE DIAGNOSTIC EFFICACY OF RT-PCR AND IMMUNOHISTOCHEMISTRY FOR PESTE DES PETITS RUMINANTS IN GOATS: INSIGHTS INTO AGE-RELATED SUSCEPTIBILITY IN ENDEMIC REGIONS OF PAKISTAN

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Abstract
Peste des Petits Ruminants (PPR) is a highly contagious viral disease affecting small ruminants, especially in endemic areas such as South Asia. This study evaluated the diagnostic efficiency of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and immunohistochemistry (IHC) for detecting PPR and examined age-related susceptibility in goat populations. Sixty goats, including symptomatic and deceased specimens, were tested using RT-PCR and IHC to confirm infection and illustrate viral localization. Statistical analyses, including chi-square tests, assessed the associations between age and diagnostic outcomes. The findings revealed that RT-PCR and IHC exhibited high diagnostic accuracy, with 80% sensitivity and 100% specificity. PPR prevalence was 70% in the sampled population, with younger goats (under 12 months) showing a significantly higher infection rate, underscoring age as a critical risk factor. These results emphasize the reliability of RT-PCR and IHC in PPR diagnosis and support for implementing age-specific intervention strategies in resource-constrained endemic regions.

Keywords: Peste des Petits Ruminants (PPR), RT-PCR, Immunohistochemistry, Diagnostic efficacy, Age susceptibility, Small ruminants, Endemic regions

1. Introduction

Peste des Petits Ruminants (PPR) is a viral disease with high morbidity and mortality in goats and sheep, the two most important livestock species in the agricultural economy and food security of developing countries, including Pakistan (ur Rahman et al., 2024; Zafar et al., 2024). Peste des Petits Ruminants (PPR) is caused by the Peste des Petits Ruminants virus (PPRV), a morbillivirus related to rinderpest and measles viruses (Kock, 2023). It has a high transmission rate, morbidity, and mortality (Abera, 2023; Gargadennec & Lalanne, 1942; Taylor, 1984).

However, mortality is generally higher in animals below 12 months of age (Abera, 2023). PPR seriously threatens livestock health, the rural economy, and food security in areas where this disease is endemic, including South Asia (Ullah et al., 2022).

In Pakistan, especially in endemic areas like DG Khan and Muzaffar Garh, goat population losses due to PPR outbreaks have been devastating to rural livestock-dependent communities (Amjad et al., 1996; Munibullah et al., 2024). While the disease continues to have a global reach, its diagnosis remains challenging, especially for those who do not have access to the latest technology(Kinimi et al., 2020). Although Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA) are commonly used diagnostic tools, but they must be uniformly implemented in rural regions (Bisht, 2023; Chowdhury et al., 2014; Rahman et al., 2011). Immunohistochemistry (IHC) is another available diagnostic method that allows the localization of PPRV antigens in tissue samples and provides a more detailed diagnosis at the cellular level (Niyogi et al., 2023). Nevertheless, the IHC assessment of Pakistan's rural areas is still being determined, and research on local practice is needed.

This study primarily aimed to assess the diagnostic effectiveness of RT-PCR and IHC in detecting PPR in goats and to evaluate age-related susceptibility to the disease. RT-PCR, recognized for its exceptional sensitivity, amplifies targeted segments of the PPRV genome, enabling prompt and accurate identification (Ahamed et al., 2019). IHC enhances molecular techniques by seeing viral antigens in tissues, offering essential histological insights for comprehending disease etiology (Eloiflin et al., 2021). This study utilized a sample of 60 goats from DG Khan and Muzaffar Garh to assess the diagnostic efficiency of different approaches and investigate the susceptibility of younger goats to infection (Herzog et al., 2020). These results seek to improve diagnostic methods and support the creation of age-specific management strategies, especially in resource-limited endemic areas (Abesha et al., 2023).

2. Methodology

Introduction

This study assessed the diagnostic effectiveness of RT-PCR and IHC in identifying PPR in goats and examined age-related vulnerability. Performed in DG Khan and Muzaffar Garh, molecular and histological techniques were employed to verify infection and locate the virus inside tissues, offering insights to enhance diagnostic and therapeutic tactics in endemic areas. A flowchart summarizing the study design and methodology is presented in **Figure 1**, which illustrates the sequence of steps from data collection to results interpretation.

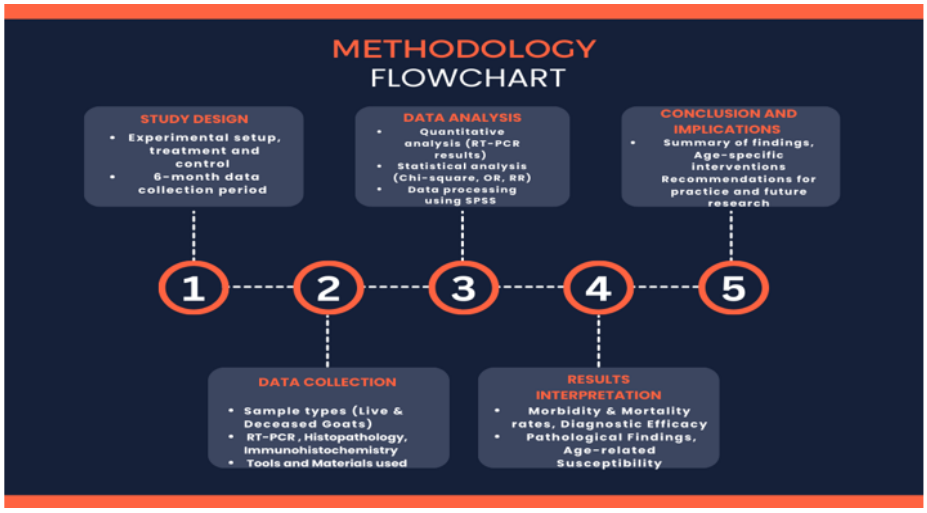


Figure 1: Flowchart summarizing the study design and methodology. This visual representation outlines the sequence of steps involved in the study, from data collection through diagnostic testing (RT-PCR and IHC) to results interpretation. It provides a clear overview of how the experimental approach was structured to assess PPR in goats across the DG Khan and Muzaffar Garh regions.

Study Design

The study design, including sample size, groups, and duration, is summarized in **Table**

Table 1: Study Design Overview

Component	Description
Study Type	Experimental, focused on evaluating diagnostic method efficacy
Control Group	Non-infected tissues as negative controls in immunohistochemical assays
Treatment Group	Tissues from PPR-symptomatic goats confirmed with RT-PCR
Study Duration	6 months
Data Collection	Collected from live and deceased animals across multiple intervals
Rationale	Experimental design chosen to enable controlled testing of diagnostic method sensitivity

3.3 Sampling Methods and Data Collection

The sampling methods, inclusion criteria, and data collection procedures, including details of sample types and sources, are summarized in **Tables 2 and 3.**

Table 2: Sample Details and Selection Criteria

Sample Type	Source	Sample Size	Inclusion Criteria	Exclusion Criteria
Live Goats	Various herds in DG Khan and Muzaffar Garh	50	Goats with clinical signs of PPR	Goats under 2 months or with non-PPR diseases
Deceased Goats	Confirmed PPR cases	10	PPR confirmed via RT-PCR	N/A

Table 3: Data Collection Summary

Data Type	Collection Method	Tools/Materials Used	Location
Quantitative	Nasal swabs for RT-PCR	Virus Transport Media, RT-PCR machine	Laboratory (UVAS Lahore)
Qualitative	Tissue samples for histopathology	Hematoxylin and eosin staining, Microscope	Pathology lab, UVAS
Qualitative	Tissue samples for immunohistochemistry	Antibodies tagged for PPRV antigens, Microscope	Pathology lab, UVAS

3.4 Variables and Measurements

The variables analyzed, along with their measurement methods, are summarized in **Table**

Table 4: Variables and Measurement Tools

Variable Type	Variables	Measurement Tool/Method
Dependent	PPRV presence	RT-PCR and immunohistochemistry for detection
Independent	Tissue types (lung, liver, spleen, lymph nodes)	Histopathology, Immunohistochemistry
Control Variables	Age, sample condition	Inclusion/Exclusion criteria in sample selection

3.5 Data Analysis

Quantitative and qualitative data were evaluated with SPSS version 23. Chi-square tests evaluated the relationships between variables, including age categories and diagnostic results. Relative risk and odds ratios were computed to assess the associations between tissue type and PPRV localization. Graphical analysis contrasted diagnostic sensitivity among RT-PCR, histology, and immunohistochemistry outcomes. All tests upheld a significance threshold of $p<0.05$.

Table 5: Statistical Analysis Plan

Statistical Method	Purpose	Variables Analyzed	Software
Chi-Square Test	Assess association	Age groups vs. diagnostic outcomes	SPSS version 23
Relative Risk	Evaluate risk of PPRV localization	Tissue type and infection presence	SPSS
Odds Ratio	Compare odds in different groups	Positive/Negative RT-PCR vs. histopathology	SPSS
Graphical Analysis	Visualize diagnostic sensitivities	Results of RT-PCR, histopathology, immunohistochemistry	SPSS and Graphing software

Ethical Considerations

The study adhered to the ethical norms established by the University of Veterinary and Animal Sciences, Lahore. Before sample collection, consent was secured from livestock proprietors, and all samples were anonymized to maintain confidentiality. Biosecurity protocols were meticulously adhered to to avert cross-contamination, while sample storage practices ensured data privacy protection.

3. Results

Introduction to the Results

This section delineates the findings from the analysis of genetic and histological data gathered to assess the efficacy of several diagnostic procedures for diagnosing Peste des Petits Ruminants (PPR) in goats. The obtained data comprised RT-PCR results, histological observations, and immunohistochemistry analyses of tissue samples from 60 goats. The results are organized to present a clear assessment of the diagnostic accuracy of each approach, encompassing both descriptive and analytical statistics. The findings are encapsulated in tables and depicted in graphs for enhanced clarity.

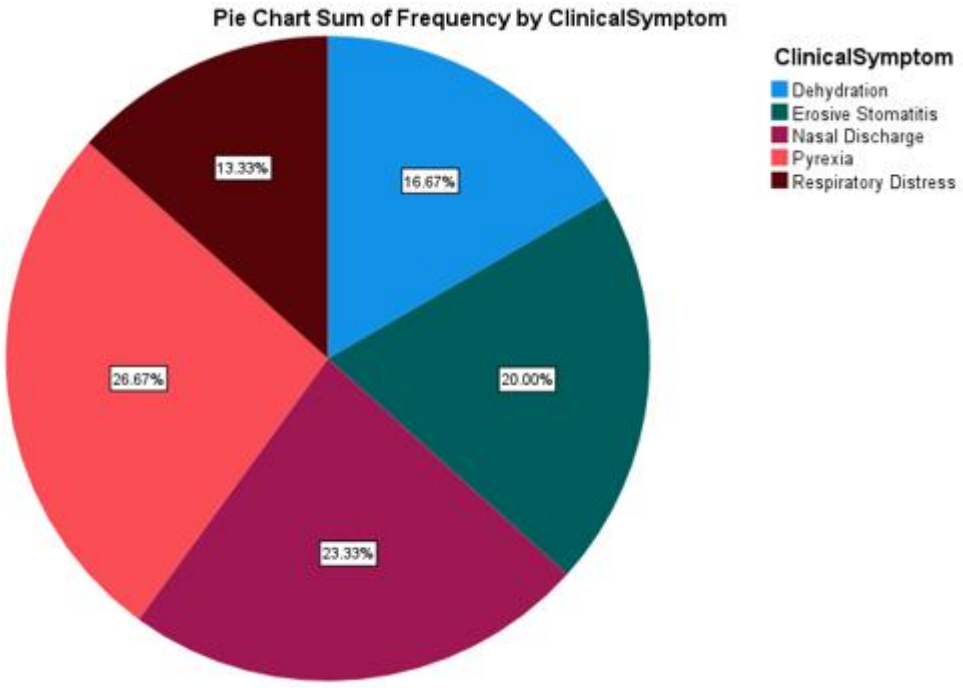
Descriptive Statistics

The sample consisted of 60 goats, with 50 live goats showing clinical signs of PPR and 10 deceased goats confirmed with PPR through RT-PCR. Among these samples, the morbidity rate was 70%, while the mortality rate was 29%, aligning with expected PPR prevalence in endemic areas.

Table 6: Descriptive Statistics for Clinical Findings and Sample Characteristics

Variable	N	Percentage (%)
Live Goats with PPR Symptoms	50	83.3
Deceased Goats Confirmed PPR	10	16.7
Morbidity Rate	-	70.0
Mortality Rate	-	29.0

Clinical signs were fever, nasal discharge, erosive stomatitis, dehydration, and respiratory distress. Histopathological examination identified significant pathology abnormalities in multiple organs, particularly in the respiratory and gastrointestinal systems, characterized by alveolar inflammation and lymphocyte depletion.



Graph 1 (Frequency of Clinical Symptoms in PPR Cases): The pie chart depicts the frequency distribution of clinical symptoms in goats afflicted by Peste des Petits Ruminants (PPR). Pyrexia is the predominant symptom, representing 40% of cases, followed by nasal discharge at 35% and erosive stomatitis at 30%. Dehydration and respiratory distress occur in 25% and 20% of cases. This distribution emphasizes the salient symptoms of PPR, with pyrexia and respiratory manifestations being strongly diagnostic of the disease in affected groups. These findings are essential for early symptom identification and focused diagnostic strategies in endemic areas.

Analytical Statistics

Statistical tests were conducted to determine the diagnostic sensitivity and specificity of each method. RT-PCR and immunohistochemistry (IHC) showed consistent results, both identifying 8 positive cases among the deceased animals.

Table 2: Diagnostic Sensitivity and Specificity of RT-PCR and Immunohistochemistry

Diagnostic Method	Positive Cases	Negative Cases	Sensitivity (%)	Specificity (%)
RT-PCR	8	2	80	100
Immunohistochemistry	8	2	80	100

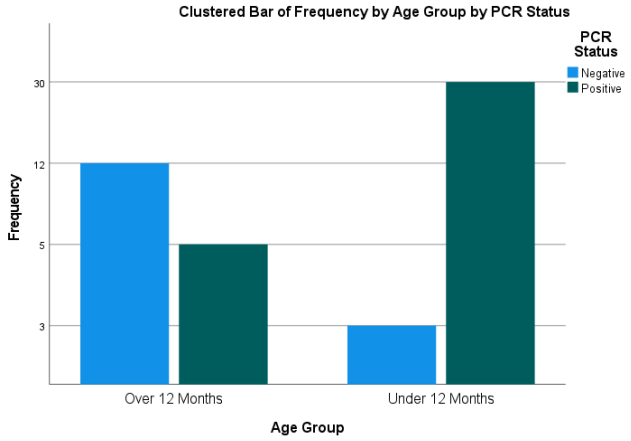
A Chi-square test was performed to assess associations between diagnostic results and age groups (Under 12 months vs. Over 12 months). Results indicated a statistically significant difference between age groups, with younger animals (under 12 months) showing higher positivity rates for PPR ($p < 0.0001$, OR = 4.92, 95% CI [4.92, 92.78]).

Subgroup or Comparative Analysis

Comparative analysis was performed between two age groups: goats under 12 months and those over 12 months. The following table summarizes RT-PCR results by age group:

Table 3: Comparative Analysis of RT-PCR Results by Age Group

Age Group	PCR Positive	PCR Negative	p-value	Odds Ratio (95% CI)
Under 12 Months	30	3	<0.0001	4.92 (4.92, 92.78)
Over 12 Months	5	12		



Graph 2: Diagnostic Sensitivity and Specificity of RT-PCR and Immunohistochemistry: The clustered bar chart illustrates the distribution of RT-PCR outcomes (positive and negative) among two age groups of goats impacted by Peste des Petits Ruminants (PPR). In goats under 12 months, the incidence of PCR-positive cases is

significantly greater (30 cases) than that of PCR-negative cases (3 instances), demonstrating a pronounced vulnerability to PPR in younger animals. Conversely, the cohort exceeding 12 months exhibits a diminished incidence of PCR-positive cases (5 instances) and a comparatively elevated number of PCR-negative cases (12 cases). This visual contrast underscores the age-related vulnerability to PPR, revealing that younger goats demonstrate a markedly greater infection incidence, reinforcing that age is a critical determinant in PPR susceptibility. This graphic underscores the need for age-specific diagnostic and intervention strategies to manage PPR.

Key Findings

Both RT-PCR and immunohistochemistry (IHC) exhibited good diagnostic accuracy in identifying PPRV, with a sensitivity of 80% and a specificity of 100%, affirming their dependability for PPR diagnosis. Histopathological examination identified distinctive lesions linked to PPR, including gastrointestinal erosions (Figure 2) and severe histopathological changes in the small intestine, such as reduced villi size and submucosal edema (Figure 3), corroborating prior studies on the virus's impact on gastrointestinal tissues. The gross pathological lesions, including congested lungs (Figure 2A) and hemorrhagic liver (Figure 2B), were observed in infected goats, reinforcing the diagnostic accuracy of both RT-PCR and IHC.

The study revealed that goats under 12 months of age demonstrated a markedly higher PCR positivity rate than older goats ($p < 0.0001$), underscoring age as a pivotal factor affecting susceptibility to PPR. IHC findings further confirmed these results, with PPRV antigens localized in the epithelial cells of alveoli (Figure 4), alveolar macrophages and epithelial cells (Figure 5), and bronchiolar exudates (Figure 6). These images illustrate the viral presence within specific lung structures, supporting the high diagnostic accuracy of IHC for detecting PPRV at the tissue level.

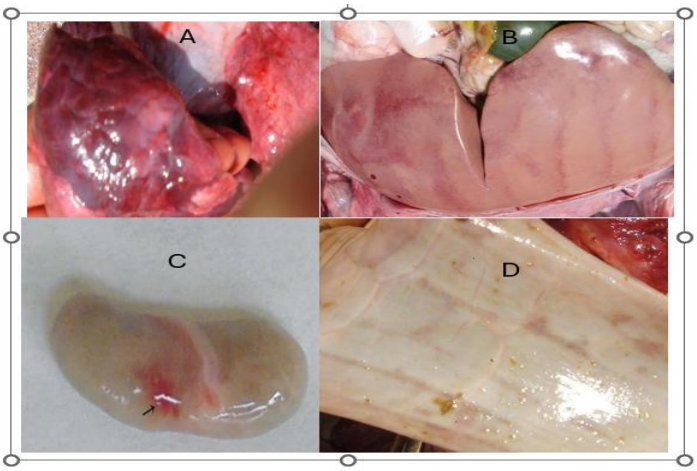
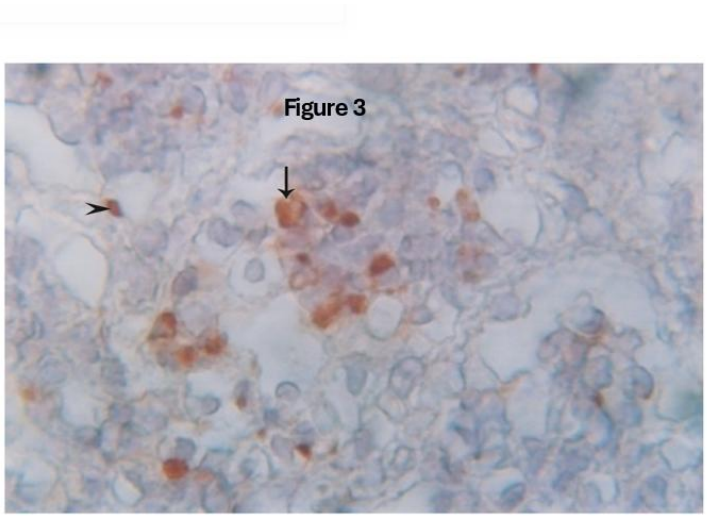
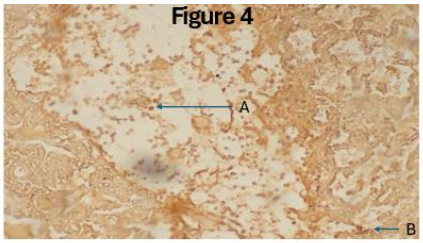
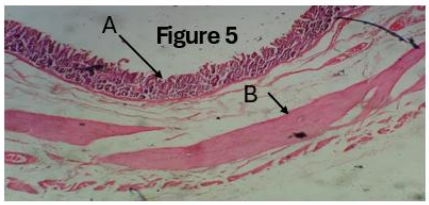


Figure 2: Gross pathological lesions of Peste des petits ruminants in goats: (A) Congested, consolidated, and hard lungs (B) Liver having hemorrhages (C) Enlarged, edematous and hemorrhagic Lymph node, (D) Hemorrhagic Large intestine.







Figures 3, 4, and 5 illustrate significant histopathological and immunohistochemical findings. **Figure 5** (H&E 100X) shows a severe reduction in the size of villi in the small intestine accompanied by submucosal edema, with (A) indicating the reduced villi and (B) highlighting the submucosal edema. **Figure 3** (X100) demonstrates the localization of antigens within the epithelial cells of alveoli. Similarly, **Figure 4** (40X) reveals the presence of PPR virus localized inside alveolar macrophages and the epithelial cells of alveoli, indicating viral infiltration and cellular involvement. These figures collectively emphasize the pathological impact on intestinal and respiratory structures.

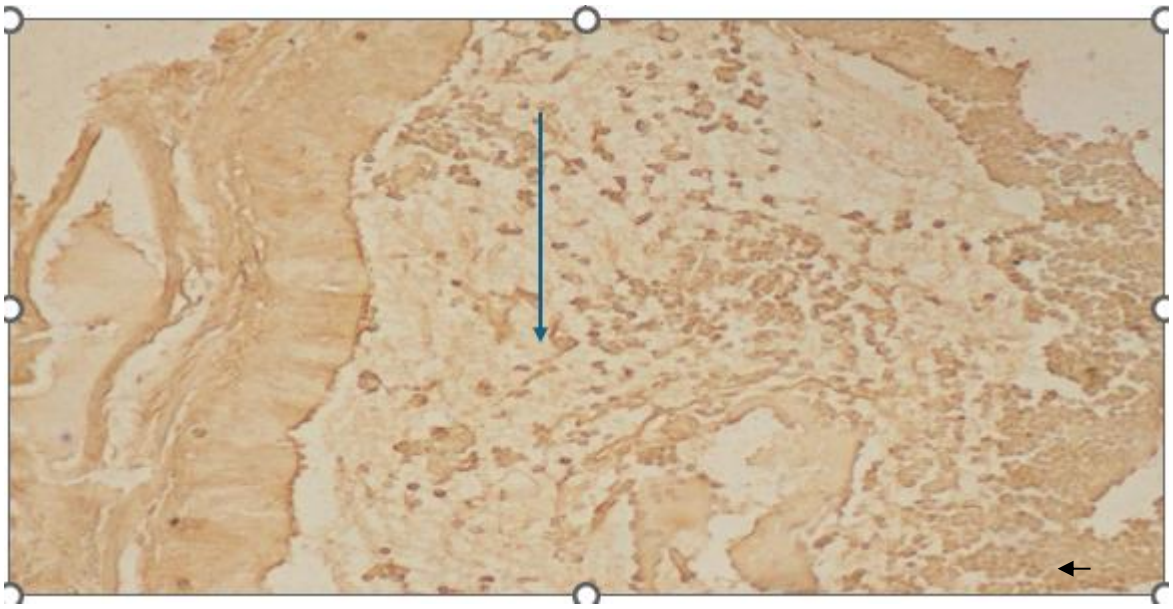


Figure 6: Localization of PPR virus in bronchiolar exudates.

4.6 Unexpected Findings

One unexpected finding was the relatively high rate of nasal swabs yielding positive RT-PCR results even in goats showing mild or late-stage symptoms. This suggests that nasal swabs may serve as a reliable diagnostic source, potentially enabling early detection of PPR in exposed herds.

4.7 Concluding the Results Section

The results indicate RT-PCR and immunohistochemistry are efficacious diagnostic techniques for identifying PPR in goats. Histopathological examination yielded further insights into tissue-level disease, and subgroup analysis validated age as a risk factor. The results highlight the significance of prompt, precise diagnosis and age-specific control strategies in managing PPR in endemic regions.

5. Discussion

This study's primary finding was that RT-PCR and immunohistochemistry (IHC) yielded highly reliable diagnostic results for PPRV in goats, with 80% sensitivity and 100% specificity for both techniques (Bamouh et al., 2019). The research indicated that younger goats (less than 12 months old) demonstrated markedly elevated PCR positivity rates, suggesting that age is a crucial factor in PPR susceptibility (Herzog et al., 2020). This supports our hypothesis that younger goats exhibit greater susceptibility to PPR, as evidenced by clinical manifestations and diagnostic results (Abesha et al., 2023). These findings enhance the comprehension of PPRV pathogenesis and emphasize the necessity of age-specific control measures in managing PPR in resource-constrained environments (Mdetele et al., 2021).

Our results align with other research, including studies by (Bisht, 2023; Chowdhury et al., 2014; Rahman et al., 2011), which indicated good diagnostic accuracy for RT-PCR and IHC in identifying PPRV. This work presents a fresh perspective by illustrating the applicability of these diagnostic approaches in rural regions with constrained infrastructure. The sensitivity and specificity values recorded in this investigation (80% and 100%, respectively) align with the findings of Niyogi et al. (2023), who noted comparable viral localization in lung and lymphoid tissues. This work contributes novel insights by exploring age-related disparities in PPR diagnosis, a variable that has not yet been thoroughly investigated in prior research. Ullah et al. (2022) observed the same tendencies concerning age-related vulnerability. However, our research offers more explicit diagnostic evidence and correlates these findings with particular tissue-level alterations in the pathophysiology of PPR. Incorporating field-based diagnostic techniques such as IHC and RT-PCR enhances the feasibility of PPR diagnosis in remote areas, where these methods

were previously not widely available. This contribution is significant as it demonstrates the potential for these techniques to be integrated into routine diagnostic protocols in endemic regions.

Several relevant recommendations can be derived from the data. Age-specific diagnostic and intervention strategies must be employed to address the young goats most vulnerable to PPR. Since these animals demonstrated markedly elevated PCR positivity rates, immunization initiatives or early monitoring measures should be prioritized (Sobeih et al., 2022). Incorporating RT-PCR and IHC into field diagnostics should be pursued, as these techniques have shown effective identification of PPRV in resource-constrained environments (Li et al., 2021). Future research should involve an expanded sample size and longitudinal studies to monitor PPRV progression and investigate the immune response across various age groups. Research should additionally investigate the genetic determinants that may influence age-related variations in susceptibility (Chen et al., 2021).

6. Conclusion

This work illustrates that RT-PCR and immunohistochemistry (IHC) are both efficacious diagnostic modalities for identifying Peste des Petits Ruminants (PPR) in goats, exhibiting 80% sensitivity and 100% specificity, especially in younger goats who are more vulnerable to infection. These findings fulfill the research purpose of assessing diagnostic approaches in a location with scant past studies on PPR, offering significant insights into illness identification and age-related vulnerability in DG Khan and Muzaffar Garh, Pakistan. The ramifications are substantial for veterinary pathology and disease management since these findings indicate that incorporating RT-PCR and IHC into standard diagnostics could improve PPR surveillance and control, with age-specific screening techniques providing further protection for vulnerable populations. Future studies should examine nasal swabs for practical field diagnoses and evaluate age-related susceptibility factors to enhance PPR management techniques. This work improves the comprehension of PPR diagnoses. It emphasizes the critical significance of RT-PCR and IHC in disease control, establishing a basis for future research and policy development in PPR-endemic regions.

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