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# Comparative pathogenesis of Ethiopia/Habru/2014 Lineage-IV peste des petits ruminants virus in goats and cattle

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## Abstract

**Background** Peste des Petits Ruminants (PPR) is a highly contagious viral disease primarily affecting goats and sheep, with clinical manifestations ranging from peracute disease to subclinical infection, particularly in atypical hosts such as cattle. The role of atypical hosts such as cattle to the spread of PPR remains controversial, with conflicting reports in the literature. Despite its worldwide significance, considerable knowledge gaps exist regarding the pathogenesis and clinical progression in both primary and atypical hosts. This study aimed to elucidate the tissue tropism, pathogenesis, virus shedding, clinical progression, and pathology associated with experimental PPR virus infection in indigenous goats and cattle. To this end, 32 animals—16 goats and 16 cattle—were intranasally inoculated with the Ethiopia/Habru/2014 Lineage-IV strain of the PPR virus followed by detailed clinical evaluations and systematic sampling at pre-established intervals to assess serological conversion, viral shedding, and the pathogenesis of the infection across both species.

**Results** The results show that goats exhibited typical clinical signs 4 days post-inoculation, with seroconversion by day 6 and early detection of viral RNA in swabs and tissues by day 3 and virus isolation starting day 4. In contrast, cattle exhibited minimal clinical signs, with seroconversion occurring at day 8 with viral RNA detected in tissue samples at day 4 and virus isolation starting day 6 in tissues and in a single nasal swab at day 8. Clinical scores and tissue positivity rates significantly differed between goats and cattle ($P = 0.007$ and $P < 0.001$, respectively). While goats exhibited expected gross and histopathological lesions, cattle showed only nonspecific lesions.

**Conclusions** Together, our findings highlight the importance of comparative pathology studies for better understanding virus dynamics and transmission pathways that may help inform more effective PPR control programs. Future research should explore the pathogenesis of different PPRV lineages in cattle, assessing variations in disease progression and potential for epidemiological impact.

**Keywords** Cattle, Comparative pathology, Goats, Disease pathogenesis, Experimental, PPR, PPRV Lineage-IV

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## Background

Peste des petits ruminants (PPR), a highly contagious viral disease primarily impacting goats and sheep, was initially identified in West Africa in 1940's [16]. It has since become widespread, affecting regions across Africa, the Middle East, and Southeast Asia [4], with a global presence in over 70 countries and territories [41]. The disease poses a significant threat to subsistence farmers, causing substantial economic losses due to its elevated morbidity and mortality rates [21]. Outbreaks of PPR not only disrupt the livelihoods of livestock owners but also have far-reaching consequences on food security and socio-economic activities, leading to a considerable disruption in affected regions [1].

PPR exhibits various clinical manifestations, including peracute, acute, subacute, and subclinical forms contingent upon predisposing factors and the virulence of the infecting virus types [8, 28]. The peracute manifestation results in severe symptoms such as depression, anorexia, abortion in pregnant animals, and often leads to death [2]. The acute form is characterized by a sudden onset of symptoms like depression, pyrexia, discharge from the eyes and nose, inflammation of mucous membranes, eyelid matting, mouth inflammation, diarrhea, coughing, and foul breath, ultimately culminating in death [4, 32, 38]. Subacute cases present milder clinical signs, resulting in lower mortality rates, with animals typically recovering within 10–14 days, a pattern more prevalent in sheep [28]. Large ruminants like cattle may display a subclinical form [3, 35, 36], where the virus is eliminated without apparent clinical symptoms, accompanied by robust neutralizing antibody responses [28].

In highly susceptible hosts (goats and sheep) the tissue tropism, pathogenesis and disease expression after PPRV infection is associated with infection of immune and epithelial cells via Signaling Lymphocyte Activation Molecule (SLAM) and nectin-4 receptors, respectively [7, 30, 35]. However, the role of atypical hosts, such as cattle, in PPRV infection remains a subject of debate with conflicting findings in the literature. Sen et al. [36] report that cattle may have prolonged viral presence in blood and peripheral blood monocyte cells and are a potential source of PPRV transmission [36]. In contrast, other groups classify cattle as dead-end hosts and report seroconversion and short-term low levels of PPRV RNA in oronasal swab samples [10, 35]. The inconsistency in results from field and experimental studies can be attributed to various factors, including differences in methods, strains, breeds, virus doses, and environmental conditions.

Despite the global impact of PPR, there is a paucity of knowledge regarding its pathology and clinical progression, particularly in atypical host species like cattle. Questions regarding the potential role of species other than sheep and goats, particularly cattle, in PPRV persistence remain unanswered. Recognizing the importance of understanding PPRV in both target and atypical hosts, a more comprehensive examination becomes imperative to establish effective surveillance and control measures.

In light of these knowledge gaps and the conflicting results reported in previous studies, the present research investigated PPRV tissue tropism, pathogenesis, shedding, clinical course, and comparative pathology in both goats and cattle. The findings provide important insights on disease progression and virus shedding patterns from various sites over intervals in both primary and atypical (cattle) hosts. These insights may be used to better guide decisions about the future development of more effective PPR surveillance and control strategies.

## Material and methods

### Experimental design and procedure

Indigenous goats (Central and Western Highland breeds) and cattle (Arsi breed), aged between one to two and a half years, were acquired from North Shoa, Ejere, and Degem animal markets, located within a 200-km radius of Addis Ababa, Ethiopia. These areas are recognized for being relatively free of PPR. Selection criteria included age, apparent health, breed authenticity, and no history of vaccination against PPR. Age verification was based on dentition [40] and corroborated by information from the sellers. Beyond visual inspection and temperature measurement, no further health assessments were conducted at purchase. The animals were then transported to the Animal Health Institute's (AHI's) experimental facility. Upon arrival, competitive enzyme-linked immunosorbent assay (c-ELISA) testing (ID.Vet, Grabels, France) was performed to confirm their seronegative status for PPR. Following a 10 to 15-day acclimatization period, during which their health was continuously monitored, the animals were ear-tagged and divided into two groups: 16 goats (Group A) and 16 cattle (Group B). Comprehensive daily evaluations of rectal temperature and clinical signs were conducted, alongside veterinary care as detailed in Additional file (AF) Text AF1. The overall study design is elaborated in Text AF2.

For the virus infection, we utilized the PPRV/Ethiopia/Habru/2014 isolate, Lineage-IV (GenBank Accession Number ON110960), cultured in Vero Dog Slam (VDS) cells at AHI. The virus strain was taken from a female goat displaying typical clinical signs of PPR in Habru area, Ethiopia. The virus was isolated and propagated up to the fourth passage using Vero Dog Slam (VDS) cells. Detail information on the strain can be found in published papers [19, 22]. The viral titer was determined to be $10^{5.3}$ $TCID_{50}$, (50% Tissue Culture Infective Dose) per

ml at the fourth passage (P4), with its presence confirmed through real-time reverse transcription-PCR (RT-qPCR) and observation of cytopathic effects (CPE) in virus cell culture as described in virological analysis section. Inoculation was performed using a 3 ml syringe attached to a MAD-300 intranasal mucosal atomization device (Teleflex Incorporated, Wayne, PA, USA), delivering 2 mL of the virus suspension per cattle (1 mL per nostril) and 1 mL per goat (0.5 mL per nostril), to mimic natural transmission. Care was taken to secure the animals and elevate their heads to ensure the effective administration of the inoculum and facilitate deep inhalation.

#### Clinical investigation and scoring

Following inoculation, daily examinations were conducted on all animals to identify the onset and progression of clinical signs indicative of PPR as outlined by other groups [29, 31, 32]. Prior to each sampling event, a thorough clinical assessment was performed on every animal, encompassing rectal temperature measurements and the observation of ocular and nasal discharges, mucous membrane congestion, oral lesions, diarrhea, coughing, respiratory distress, among other signs. These observations were systematically scored according to previously described criteria [31] and goats and cattle exhibiting a rectal temperature of 39.5 °C or higher were classified as febrile. In this study, we adhered to humane endpoints for virus-infected animals as outlined by Pope et al. [31], which recommend euthanasia on ethical grounds when animals reach a clinical score of 20. However, none of the infected animals in our research reached this maximum score within the set timeframe, so early euthanasia was not necessary.

#### Sampling and sample preparation

For both the goat and cattle cohorts, antemortem blood for serological analyses, swabs from the eyes, nose, and rectum, and tissue samples from sacrificed animals were systematically collected at predetermined days post-infection (dpi) to monitor for viral shedding, tissue tropism and seroconversion. Upon collection, swab samples with medium from goats and cattle were transported on ice for immediate analysis. Goats were euthanized daily, with two randomly selected starting from 1 dpi until 8 dpi, whereas two cattle were euthanized on 2, 4, 6, 8, 10, 17, 24, and 28 dpi. Swabs were stored in 1 ml Dulbecco's Modified Eagle Medium (DMEM), and postmortem tissue samples were placed in sterile falcon tubes with 5–7 ml PBS for transport, while tissues intended for histopathological examination were fixed in 10% buffered formalin.

Collected tissue samples including, retropharyngeal lymph node (LN), mediastinal LN, bronchial LN, mesenteric LN, tracheal LN, lung cranial, lung caudal, colon, jejunal and ileal Peyers patch, spleen, tongue (apex), palatine tonsil, nasal mucosa, and 3rd (third) eyelid. These samples allocated for RT-qPCR and viral culture analysis were preserved in 5-7 ml phosphate-buffered saline (PBS) and stored at -80 °C. These samples were homogenized upon thawing followed by centrifugation and the resultant supernatant was used for both RT-qPCR testing and culture. RT-qPCR was performed on swab and tissue samples collected during the study and specimens that were positive underwent virus isolation in VDS cells to further confirm the presence of viable virus. Concurrently, for serological assessment, 5–7 ml of blood was collected from the experimental animals into plain vacutainer tubes at predetermined intervals post-infection.

#### Virological analysis (PCR, Ag-ELISA, virus isolation)

The detection of PPRV antigen was conducted using real-time RT-qPCR and immunocapture-ELISA (Ic-ELISA) as described below. RT-qPCR positive samples were further subjected to virus isolation. Despite not being fully validated with cattle samples, the PPR Ic-ELISA was utilized to examine nasal and rectal swabs from both species.

For RT-qPCR, in brief, viral RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) per manufacturer's protocol, and used in an RT-qPCR assay targeting the N gene of PPRV using the specific primers and probe described by [6]. The reaction mixture comprised 10 µl of Express Universal SuperScript mix, 2.0 µl of Express SuperScript enzyme mix, 0.4 µl of ROX reference dye (Invitrogen, Thermo Fisher Scientific), 0.8 µl of each primer (forward and reverse), 0.4 µl of the PPR probe, 2.6 µl of RNase-free water, and 3 µl of the extracted RNA, totaling a 20 µl reaction volume. Amplification was performed using an Applied Biosystems 7500 FAST thermal cycler with reverse transcription at 50 °C for 15 min, followed by DNA polymerase activation at 95 °C for 20 s, and 45 cycles of denaturation at 95 °C for 3 s, and annealing/extension at 60 °C for 30 s, based on the protocol by [6].

For virus isolation, VDS cells were prepared by washing with PBS and then inoculated with 100-200 µl of supernatants from ocular, nasal, and rectal swabs or processed tissue, previously homogenized and centrifuged at 1650 g for 20 min at 4 °C. These cells, with over 70% confluence in 24-well plates, underwent a pre-adsorption process, followed by an hour of incubation at 37 °C with 5% CO2 and 96% humidity, with intermittent agitation every 15 min to promote virus adsorption. After removing the inoculum and washing with serum-free DMEM, 500 µl of DMEM with 2% serum was added to each well. The

cultures were then observed under an inverted microscope for non-specific reactions before a final incubation of five to seven days under the same conditions to identify CPE indicative of viral replication, in line with the protocol described by [17].

For antigen detection, we used the ID Screen® PPR Antigen Capture kit from ID.Vet (Grabels, France) following the Ic-ELISA method [23]. Results interpretation was straightforward, with a sample-to-positive (S/P%) ratio below 20% indicating negative outcomes, and ratios of 20% or higher signaling positive detections according to the manufacturer's instruction.

#### Serology, postmortem examination and histological analysis

In parallel, antibody responses and baseline exposure to PPRV in serum samples from goats and cattle were assessed using the ID Screen® PPR Competition kit (ID. Vet, Grabels, France). This c-ELISA kit was designed to detect anti-PPRV-N protein antibodies [24], indicative of virus exposure. The criteria for result interpretation classified samples as positive with a competition percentage (S/N%) of 50% or less, doubtful for percentages greater than 50% but at or below 60%, and negative when exceeding 60% according to the manufacturer's instruction.

In this experiment, we employed a captive bolt stunning to render the animals unconscious before euthanasia. This approach enhances animal welfare by preventing unnecessary pain and cruelty during the process and also improves safety standards. The stunning was promptly followed by effective bleeding, resulting in euthanasia. The captive bolt pistol used was the Bolt Stunner Model KS from Karl Schermer GmbH, Germany, selected for its suitability for both goats and cattle. Gross pathology and photographic documentation of any gross tissue lesions was undertaken at postmortem examination. Subsequently, a comprehensive postmortem examination was conducted on each animal to evaluate for gross pathological changes. Tissue and lymph node (LN) inspections were meticulous, focusing on areas affected by the PPR virus. This included the buccal and nasal cavities, conjunctiva, head lymph nodes (including palatine tonsils), mesenteric regions (encompassing both LNs and bowel tract), the respiratory system (lungs and associated LNs), and visceral organs such as the liver, spleen, and kidneys. Documented changes, including any deviations in structure, morphology, and color, were systematically scored to quantify the extent of the lesions, with scoring criteria detailed in Table AF1.

Histopathological examination was performed on a total of 480 tissue samples to assess cellular changes induced by PPRV infection. Standard operating procedures (SOPs) of the AHI pathology laboratory were followed. In brief, formalin-fixed tissue samples were dehydrated in graded ethanol and clarified with xylene with the Shandon Citadel 1000 tissue processor (Thermo Fisher Scientific, Waltham, MA, USA) prior to embedding in paraffin using the TEC2900 modular Tissue Embedding Centre (Histo-Line Laboratories, Milan, Italy). Tissue sections were prepared at a thickness of 4-5 µm and stained with Hematoxylin and Eosin, were examined under light microscopy at 4x, 10x, and 40× magnifications, and histopathological lesions, were scored using a grading system from 0 (no lesions) to 4 (severe lesions) as outlined in Table AF1.

#### Data analysis and visualization

Analysis and visualization of the dataset were conducted in R statistical software (version 4.1.3), employing the ggplot2 package for generating graphical representations. To illustrate trends within the groups of inoculated animals, local regression (LOESS) smoothed curves with corresponding 95% confidence intervals, calculated through t-based approximation, were incorporated into the plots using the stat-smooth function to provide a visual summary of the collective data trends across the study duration. Comparative analyses, focusing on tissue positivity rates and clinical scoring between goats and cattle, were performed using a non-parametric, Mann–Whitney test within GraphPad Prism (version 10.1.1). To assess the concordance between different methods of sample analysis, Cohen's kappa coefficient was calculated using the psych package in R, offering a statistical measure of inter-rater agreement for categorical items. It was interpreted as per [26]. If value of kappa is "< 0", No agreement; "0–0.20", Slight agreement; "0.21–0.40", Fair agreement; "0.41–0.60", Moderate agreement; "0.61–0.80", Substantial agreement; "0.81–1.00", Almost perfect agreement. All data and R-code are provided (https://github.com/cherz4/pprv-cattle-goat-comparative-pathology).

## Results

#### Experimental infection with Lineage-IV PPRV/Ethiopia/Habru/2014 strain results in moderate clinical signs in goats but no obvious clinical signs in cattle

Goats inoculated with Lineage-IV PPRV/Ethiopia/Habru/2014 presented no obvious clinical signs until 3 dpi. From 4 dpi, fever was noted in 3 of the 10 goats, and a peak rectal temperature of 40.5 °C was noted at 6 dpi (Fig. 1A and B). Serous nasal discharge appeared in 5 of the 10 goats at 4 dpi, increasing to 6 of the 8 remaining goats at 5 dpi (Figure AF1A). Additionally, at 5 dpi, congested mucous membranes were observed in the conjunctiva of 5 of the 8 goats (62.5%), while diarrhea was observed in a single animal (12.5%) (Figure AF1B).

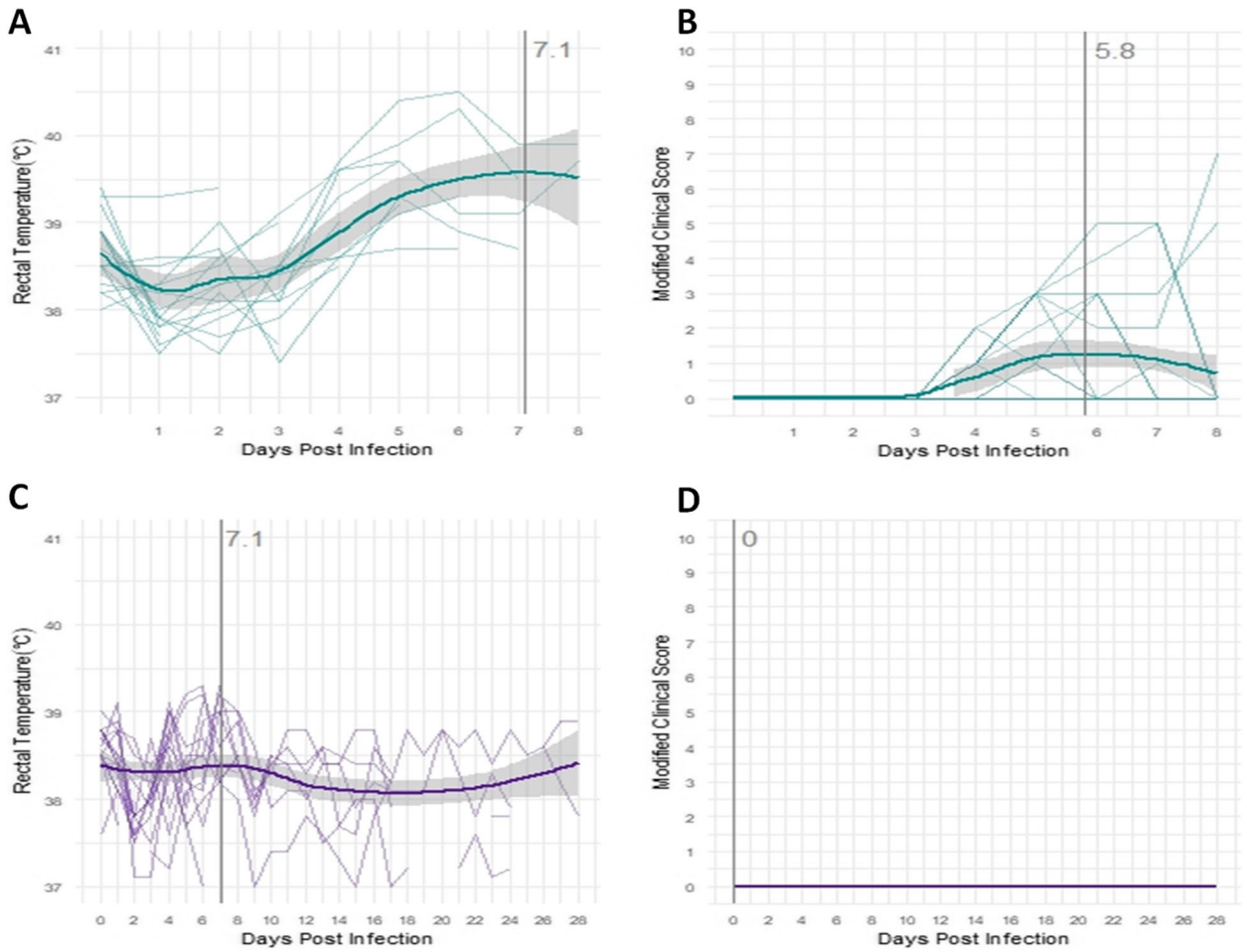


**Fig. 1** Clinical Signs and Rectal Temperature in Goats and Cattle infected with PPRV (**A**) Trends in daily rectal temperature in goats post-infection (**B**) Modified composite clinical scores in goats without rectal temperature readings. **C** Daily rectal temperatures in cattle post-inoculation, with all values remaining below the febrile threshold of 39.5 °C. **D** Composite clinical scores in cattle, indicating the absence of significant clinical signs or elevated temperatures. In all subfigures, thin lines depict individual animal data, while bold LOESS curves represent the collective pattern. The bold vertical lines in Fig. 1A, B and C denote the dpi of the peak value, while the zero value in Fig. 1D indicates no recorded clinical signs or fever

By 6 dpi, 5 of the 6 remaining goats (83.3%) continued to display serous nasal discharge, while 1 of 6 (16.7%) exhibited muco-purulent nasal discharge and diarrhea. Congested conjunctiva and coughing were noted in 2 of 6 goats. At 7 dpi, 2 of the 4 remaining goats had serous nasal discharge, and the other 2 displayed muco-purulent nasal discharge; congested conjunctiva was noted in 3 of the 4 goats.

At the conclusion of the trial (8 dpi), both remaining goats showed signs of conjunctival congestion and diarrhea. One of the goats exhibited serous nasal discharge, and the other muco-purulent nasal discharge and cough. Moreover, one animal presented with distinct oral lesions, including lesions and sloughing of the oral mucosa and gums (Figure AF1C). A detailed summary of the clinical signs observed post-inoculation in the goat cohort is provided in Table AF2.

In contrast, no clinical signs were observed following experimental infection of cattle with lineage-IV PPRV/Ethiopia/Habru/2014 strain. Rectal temperature ranged from 36.1 °C to 39.3° C, and clinical scores remained 0 through the trial (Fig. 1C and D). Significant differences in clinical observations were noted between the goat and cattle groups ($P$-value = 0.0070, Mann–Whitney test), underscoring the variability in host response to PPR virus inoculation.

### Serological responses of goats and cattle after experimental infection with PPRV/Ethiopia/Habru/2014 strain

Seroconversion in the experimental goat cohort, as detected by c-ELISA, was observed at 6 dpi in 1 of the 6 goats (16.7%), but by 7dpi all remaining goats in the cohort had seroconverted (Fig. 2A, Table 1). In cattle,

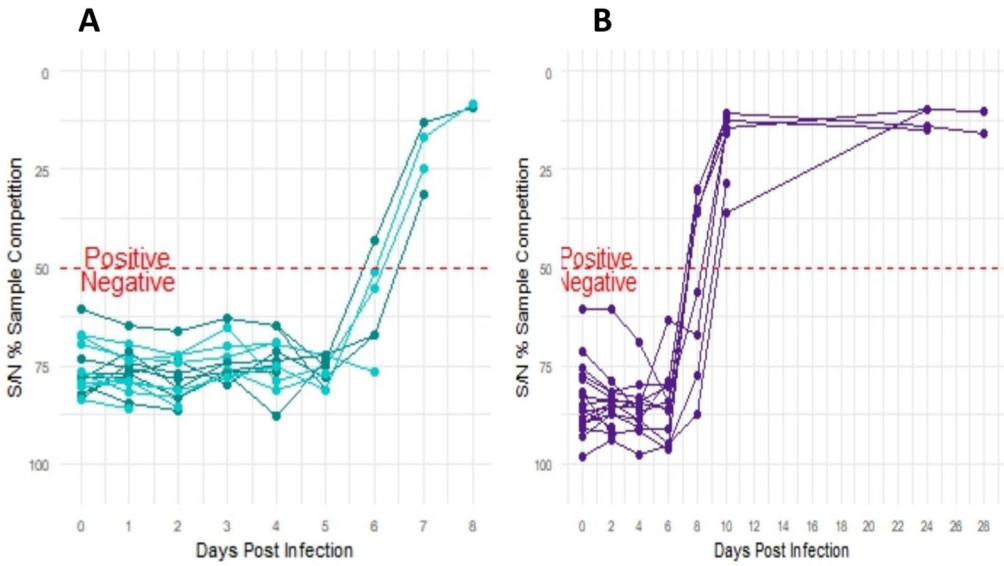


**Fig. 2** Time course of seroconversion in goats and cattle inoculated with PPR Virus highlight goats seroconverting earlier than cattle. **A** In the goat cohort, seroconversion was detected from 6 dpi onwards. Each line represents the serological response of an individual goat, with seropositivity defined by a competition percentage below 50%. The dashed red line denotes the cutoff threshold for a positive test result. **B** Among cattle, seroconversion commenced from 8 dpi. Similar to goats, individual cattle responses are shown by lines, with the cutoff for seropositivity marked by a dashed red line at the competition percentage of less than 50%

seroconversion was detected in 6 of 10 animals at 8 dpi, and in all cattle from 10 dpi (Fig. 2B, Table 1).

### Temporal dynamics of PPR virus antigen and RNA detection in antemortem and post-mortem samples in goats and cattle experimentally infected with PPRV/Ethiopia/Habru/2014

Within the goat cohort subjected to experimental inoculation, viral antigen in nasal swabs was first detected at 2 dpi in 1 of 14 goats. Fecal swab analyses revealed the presence of PPRV-antigen starting at 4 dpi in 1 of 10 goats. The frequency of detection in nasal swabs rose to 2 out of 12 goats by 3 dpi, escalating to 7 out of 10 goats by 4 dpi. From 5 dpi onward, nasal swabs from all goats tested positive for PPR virus antigens. The trend of antigen positivity in fecal swabs saw gradual increase from 4 dpi (1/10 goats), escalating systematically to 100% positivity by the 8th dpi (Table 1, Fig. 3).

In the cattle cohort, the emergence of PPR virus antigens in nasal swabs was noted as early as 2 dpi in 1 out of 14 cattle, with a progressive increase in detection rates, albeit with some fluctuations observed at various dpi benchmarks. Specifically, at 4 dpi, 3 out of 14 cattle tested positive; this positivity rate evolved to 6 out of 12 by 6 dpi, and reached 100% by 10 dpi and was, maintained through 17 dpi (6/6), 24 dpi (4/4), and 28 dpi(2/2). Fecal swabs in cattle revealed antigen detection beginning at 4dpi (1/14), at 6 dpi (1/ 12); at 8 dpi (1/10); at 10 dpi (3/8); at 17 dpi (4/ 6); at 28 dpi (1/2) (Table 1, Fig. 3).

The presence of PPRV-RNA was initially detected in goats at 3 dpi, with subsequent increases observed over time. Specifically, at 4 dpi, the virus was detected in 4 of 10 goats, primarily in ocular swabs. By 5 dpi, the virus was present in 6 of 8 goats, found in both ocular and nasal swabs. This trend continued, with the virus being detected in increasing numbers of goats and in various swab types up to 8 dpi (Fig. 3, Table 1).

RT-qPCR testing revealed an escalating presence of PPR virus RNA in post-mortem tissue and LN samples from inoculated goats, with no detection at 1 and 2 dpi, but a progressive increase from 3 dpi onward. Cumulative data indicated an increase in positive samples: starting with 25% at 3 dpi, it peaked at 89% by 8 dpi. Cattle, conversely, showed a singular instance of viral RNA positivity in a nasal swab at 8 dpi (eartag ID 211), with a subsequent uptick in tissue samples over time, highlighting species-specific resistance to the virus.

Viral culture results from samples primarily positive for RNA in goats confirmed active viral replication based on typical characteristic CPE, with a high culture positivity rate from 5 dpi onwards in swab (Table AF5) and tissue samples (Table AF6). In contrast, for cattle, culture positivity was considerably lower, with only one nasal swab (from animal ID 211) and a limited number of tissues yielding

**Table 1** Temporal dynamics of clinical scores, PPRV antigen and RNA detection and pathological scores in experimentally infected goats and cattle

| Species | ID | DPI | Clin Score | cELISA | AgELISA | PCR Tissue Positivity (Ct values) | | | | | | | | | | | | | | Total PCR Positive | Path Scores | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | NasMuc | Tons | 3rdEyelid | RPLN | Tongue | TrachLN | BronchLN | LungCran | LungCaud | MediLN | Spln | PP | Colon | MesLN | | Gross | Histo |
| Goats | 235 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | - |
| | 246 | 1 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | 1 |
| | 239 | 2 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | 1 |
| | 208 | 2 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | 1 | 1 |
| | 234 | 3 | 0 | - | - | - | 23.3 | - | 19.3 | - | 20.4 | - | - | - | 30.3 | - | - | - | - | 4 | 1 | 2 |
| | 200 | 3 | 0 | - | - | - | - | - | 20 | - | 20.5 | - | - | - | - | 25.6 | - | - | - | 3 | 1 | 2 |
| | 198 | 4 | 1 | - | 38.4 | 27.8 | 19.6 | 22.3 | 17.5 | - | 20.5 | 21.6 | - | - | - | 22.5 | 21.4 | - | 22.8 | 9 | 2 | 2 |
| | 195 | 4 | 0 | - | 20.7 | - | 21.1 | 20.6 | 17.7 | - | 19.3 | 18.4 | - | - | 20 | 20.6 | 26.6 | - | 21.4 | 9 | 2 | 2 |
| | 241 | 5 | 4 | - | 88.4 | 24.7 | 17.5 | 19.7 | 15.8 | 26.5 | 16.2 | 16.5 | - | - | 16.6 | 19.8 | - | 26.6 | 16.7 | 11 | 2 | 2 |
| | 209 | 5 | 1 | - | 103.1 | - | 18 | - | 18.2 | - | 20.2 | 20.2 | - | - | 20.3 | 22 | - | - | 24.2 | 7 | 2 | 2 |
| | 150 | 6 | 3 | - | 310.8 | 24.2 | 15.9 | 18.9 | 16.8 | - | 18.4 | 17.8 | - | - | 19.3 | 20.3 | 24.7 | - | 17.5 | 10 | 2 | 2 |
| | 178 | 6 | 3 | - | 302.7 | 26.5 | 20 | 27.8 | 21.4 | - | 28.2 | 20.4 | - | - | 20.5 | 21.3 | 23.9 | - | 20.8 | 10 | 2 | 2 |
| | 206 | 7 | 6 | 24.7 | 313.1 | 17.9 | - | 31.3 | 22.3 | 18.5 | 16.5 | - | - | - | - | 16 | 16.8 | - | - | 7 | 2 | 2 |
| | 196 | 7 | 5 | 31.1 | 314.2 | 24.7 | 17.7 | 19.9 | 23.4 | 20.1 | 18.3 | 15.6 | 20 | 19 | 16.7 | 15.9 | 15.7 | 18.8 | 17.6 | 14 | 2 | 2 |
| | 254 | 8 | 6 | 8.4 | 319 | 20.4 | 19.1 | 18 | 15.4 | 20.5 | 15.6 | 15.5 | - | 22.5 | 15.5 | 16.4 | 19 | 15.4 | 14.7 | 13 | 2 | 2 |
| | 253 | 8 | 8 | 9.2 | 316.7 | 19.4 | 17.8 | 17.2 | 16.1 | 18.1 | 17.2 | 16.7 | - | - | 16.5 | 16.6 | 24.7 | 15 | 15.9 | 12 | 2 | 2 |
| Cattle | 222 | 2 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | 1 |
| | 227 | 2 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | 1 |
| | 213 | 4 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | 1 |
| | 220 | 4 | 0 | - | - | - | - | - | 30.2 | - | 29.3 | - | - | - | - | - | - | - | - | 2 | 1 | 1 |
| | 221 | 6 | 0 | - | 22.8 | - | 32.5 | - | 24.5 | - | - | 26.6 | - | - | 28.6 | - | - | - | - | 4 | 1 | 1 |
| | 229 | 6 | 0 | - | - | - | 24.7 | - | 22 | - | - | 24 | - | - | 24.5 | - | - | - | - | 4 | 1 | 1 |
| | 212 | 8 | 0 | 29.8 | 22 | - | 22.5 | 24.1 | 24 | - | 29.6 | 25.4 | - | - | 26.6 | - | - | - | - | 6 | 1 | 1 |
| | 225 | 8 | 0 | 29.7 | - | - | 22.5 | 26.2 | - | - | - | 31.3 | - | - | 32.6 | - | - | - | - | 4 | 1 | 1 |
| | 217 | 10 | 0 | 28.6 | 42 | - | 29.7 | 27.7 | - | - | - | - | - | - | - | - | - | - | - | 2 | 1 | 1 |
| | 219 | 10 | 0 | 15.9 | - | - | - | - | - | 24.4 | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 |
| | 216 | 17 | 0 | 9.5 | 88.3 | - | 24.8 | - | 28.8 | - | - | - | - | - | - | - | - | - | - | 2 | 1 | 1 |
| | 226 | 17 | 0 | 9.8 | 103 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | 1 | 1 |
| | 211 | 24 | 0 | 9.9 | 29.9 | - | 24.9 | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 |
| | 214 | 24 | 0 | 14.9 | 29.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0 | 1 | 1 |
| | 223 | 28 | 0 | 10.3 | 28.8 | - | 24.7 | - | 27.6 | - | - | 26.8 | - | - | 26.5 | - | - | - | - | 4 | 1 | 1 |
| | 230 | 28 | 0 | 15.6 | 20.7 | - | - | - | - | 28.6 | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 |

"DPI", days post-infection ; NasMuc - Nasal Mucosa; Tons - Tonsil; 3rdEyelid - Third Eyelid; RPLN - Retropharyngeal Lymph Node; Tongue - Tongue (Apex); TrachLN - Tracheal Lymph Node; BronchLN - Bronchial Lymph Node; LungCran - Lung Cranial; LungCaud - Lung Caudal; MediLN - Mediastinal Lymph Node; Spln - Spleen; PP - Ileal & Jejunal Peyer's Patches; Colon - Colon; MesLN - Mesenteric Lymph Node.; "-" Undetermined with RT-qPCR CT > 35 or apparently normal Gross and Histology results in tissues, or >50% threshold (negative) results in cELISA, or<20% threshold (negative) results in AgELISA tests. "Total PCR Positive" implies the number of tissue samples out of 14 that yielded positive results for PPRV RNA at each respective time point

live virus, chiefly from the head and thoracic regions at 6 dpi (from animal ID 229) (Table 2). Figures of virus-induced CPE in a culture-positive nasal swab sample (Animal ID 211), along with a comparison figure without CPE in a negative control sample are shown in Fig. 4. For the outcomes of entire cultured cattle samples, refer to (Table AF7). The significant difference in tissue positivity rates between goats and cattle ($P<0.0001$, CI = 33.75–70.11) further illustrates the striking differences in patterns of disease and infection observed between the species.

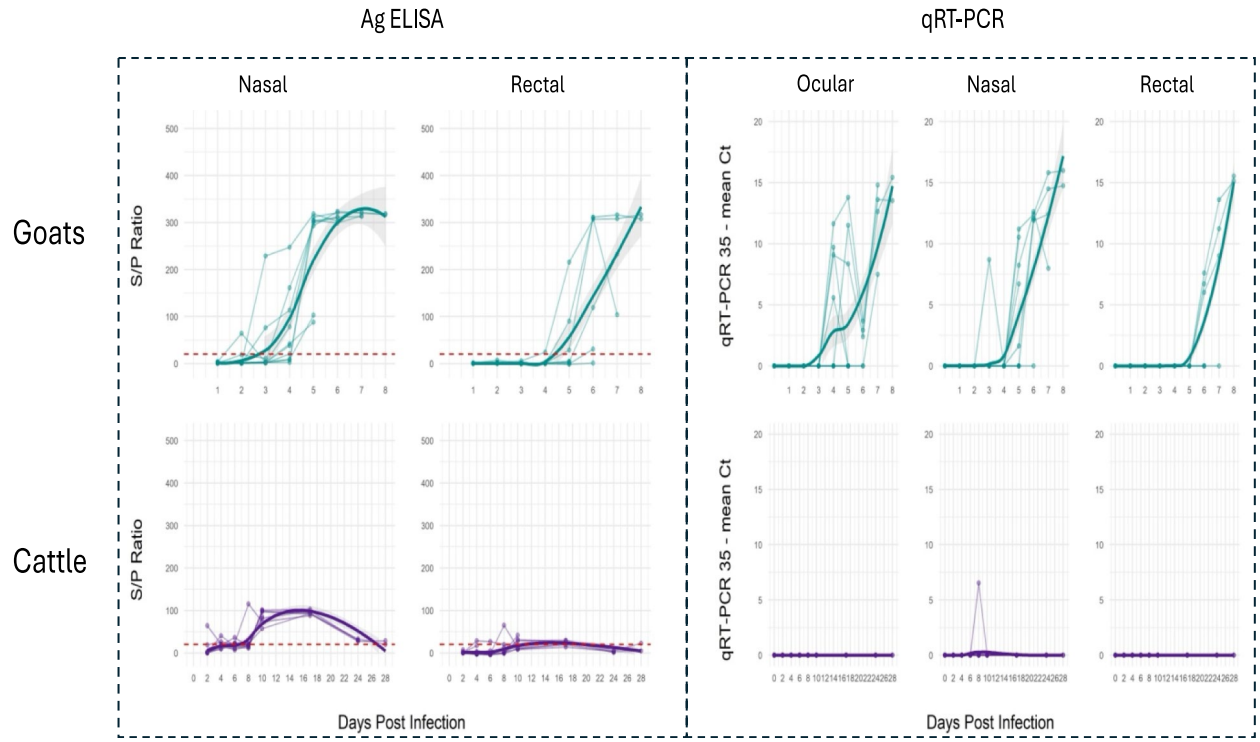


**Fig. 3** Kinetics of PPRV detection in experimentally inoculated goats and cattle. The left panels illustrate the sample-to-positive (S/P) ratio values from Ic-ELISA of the nasal, and rectal swabs, while the right panels show the cycle threshold (35-mean Ct) values from RT-qPCR of the ocular, nasal and rectal samples. The top row represents data from goats (teal color plots), and the bottom row from cattle (purple color plots). Each line depicts the virus antigen shedding pattern of individual goat or cattle, with antigen positivity defined by a percent positivity ratio of ≥ 20%. The dashed red line indicates the cutoff threshold for a positive test result. Bold lines represent smooth local regression (LOESS) curves encompassing all animals in both experimental groups

**Table 2** Observed cytopathic effect in positive cattle tissue samples[a]

| ID | DPI | Sample Type | Culture Day | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 8 |
| 229 | 6 | Retropharyngeal LN | - | + + | + + + | + + + | + + + | + + + | + + + |
| 229 | 6 | Mediastinal LN | - | - | - | + + | + + | + + | nd |
| 229 | 6 | Bronchial LN | - | - | - | - | - | + | + + |
| 229 | 6 | Palatine Tonsil | - | - | + | + + + | + + + | + + + | nd |
| 211 | 8 | Nasal Swab | - | - | + + | + + + | + + + | nd | nd |

[a] Cytopathic effect (CPE) observed in positive tissue samples from animals 229 and 211 over various culture days. Symbols indicate CPE intensity: "-" (none), "+" (mild), "+ +" (moderate), "+ + +" (strong), and "nd" (not determined). Samples from animal 229 were collected at 6 days post-infection (DPI), and from animal 211 at 8 DPI. Only positive samples are shown; tissues and swabs collected from all other animals were negative based on culture and observed CPE

The agreement between different virological analysis methods was assessed using Cohen's kappa method and was restricted to experimental goats given the low positivity rates observed in cattle. The analysis included real-time RT-qPCR, Ic-ELISA, virus culture, and c-ELISA. The findings indicated substantial agreement between Ic-ELISA and RT-qPCR tests for PPRV, with a Cohen's kappa score of 0.75. Additionally, virus culture and c-ELISA tests showed almost perfect agreement, with a Cohen's kappa score of 0.82. However, the majority of the tests exhibited fair agreement, with one test suggesting slight agreement between Ic-ELISA and c-ELISA tests. Detailed test agreement results interpreted as per [26] are provided in (Table AF4).

### Gross and histopathological lesions associated with PPR virus infection in goats and cattle

Gross and histopathological lesions associated with PPR virus infection were semi-quantitatively evaluated using

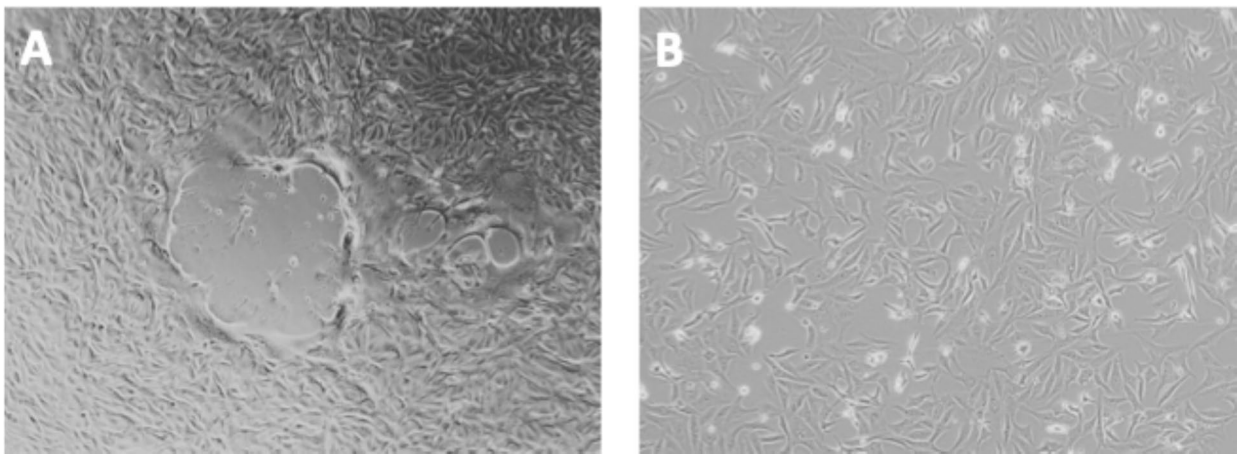


**Fig. 4** Cytopathic effects of PPRV in Vero Dog SLAM (VDS) cells. **A** VDS cells infected with PPRV isolated from a bovine nasal swab (Animal ID 211) at 8 days post-infection. The image shows pronounced cytopathic effects (CPE) characteristic of PPRV infection, including a large central syncytium formed by cell fusion. Surrounding the syncytium are elongated fibroblast-like cells and some rounded cells, indicating viral-induced cellular changes. **B** Uninfected control VDS cells showing normal monolayer morphology with typical elongated, fibroblast-like appearance and regular distribution. Images were converted from color to monochrome and minimally adjusted for brightness and contrast uniformly across the entire image using Microsoft PowerPoint. No other adjustments were made (Magnification 10x, regular tissue culture inverted microscope)

a prepared lesion scoring system (Table S1), which provided a comparative perspective on the clinical and pathological impacts of PPR virus in goats and cattle.

In brief, gross pathology findings in goats revealed a spectrum of lesions that evolved over the course of the study. Starting from 2 dpi, there was notable swelling and edema in lymph nodes, especially retropharyngeal and bronchial, along with vascular congestion in the mesenteric region and hemorrhages in the jejunal and ileal Peyer's patches. This pathology intensified by 4 dpi, with almost all lymph nodes exhibiting enlargement and hemorrhaging, and the lung lobes showing calcification in some cases. The spleen was often enlarged, and hemorrhagic enteritis became apparent. Lesions diversified further by 8 dpi, with more severe respiratory and GIT involvement.

Cattle exhibited milder, less specific gross lesions. Early signs included lymph node swelling and palatine tonsil changes, with the addition of enteritis and lung hemorrhage by 8 dpi. More prolonged disease courses in cattle revealed spleen atrophy and lung hepatization by the later stages of the study.

Histopathologically, goats displayed early lymphoid depletion and pulmonary changes, which progressed to bronchopneumonia and parenchymal necrosis in lung tissues (Figs AF2C and AF2D). By 8 dpi, severe characteristic PPRV lesions were identified, including syncytial cells and marked lymphoid depletion. Necrosis was prominent in the palatine tonsil, along with infiltrating neutrophils and syncytial cell formations, as depicted in supplemental figures (Figs AF2A and AF2B). The spleen showed hemosiderin deposits and heightened cellularity (Figs AF2E and AF2F), while broncho-interstitial pneumonia and severe hemorrhage and congestion marked the lung pathology (Figure AF2G and AF2H). Cattle, in contrast, showed more subtle histopathological changes with less frequency and severity. Lymphoid depletion was observed, but the intestinal eosinophil infiltration and mucosal necrosis noted by 10 and 24 dpi highlighted the species' distinct response to PPR virus challenge (Figs AF3A and AF3B).

Together, the gross and histopathological findings underscore the stark contrast in the pathogenesis of PPRV between goats and cattle, similar to the difference observed clinical signs between the two species.

## Discussion

Our study was designed to provide comparative insights into the pathology of PPRV lineage-IV in goats and cattle, with a specific focus on viral shedding patterns and the potential epidemiological role of cattle. Our findings of live PPR virus recovered from bovine samples adds a new perspective to our understanding of PPRV dynamics. While these findings challenge the established notion that cattle are mere dead-end hosts, and improve our understanding of PPRV pathophysiology and epidemiology, they require further study before generalization to other lineages or geographic regions beyond the lineage-IV PPRV circulating in Ethiopia.

In this study, the onset of pyrexia in goats starting from 4 days post-inoculation (dpi) is consistent with previous experimental research by [14, 29, 39], which reported

similar early fever responses in caprine subjects infected with different strains of PPRV. This early fever manifestation also corresponds closely with clinical patterns documented during PPR field outbreaks as described by [32], suggesting that the experimental model may reflect natural disease progression. In contrast, the lack of noticeable clinical signs post-inoculation in cattle are consistent with findings from studies such as those by [10, 35], which also reported minimal to no clinical manifestations in bovine hosts, supporting the hypothesis that cattle may exhibit a subclinical course of PPRV infection, potentially acting as silent carriers of the virus. This has raised a concern that such asymptomatic carriers may play a critical but understudied role in the epidemiology of PPR, particularly in mixed farming systems where goats and cattle coexist, and where silent infection in cattle could go unnoticed while contributing to disease dynamics.

The timelines for seroconversion observed in this study suggest an earlier immune response post-infection i.e. 6 and 8 dpi in goats and cattle respectively, when compared to prior studies [14, 34, 35] that reported sero-conversion at 7 and 10 dpi in goats and cattle, respectively. This somewhat accelerated seroconversion could be attributed to several factors including the specific PPRV strain used, which was Lineage-IV, the species of the host animals involved, and the methodical timing of the sampling schedule. Such differences highlight the complex interplay between the viral genetics and the host's immune system, and they underline the necessity to consider these variables when designing control strategies and diagnostic approaches for PPRV.

The RT-qPCR analyses performed on clinical and tissue samples from caprine and bovine hosts suggest a pronounced viral RNA shedding pattern in goats, with peak periods (5–8 dpi's) of shedding aligning with those previously reported by [18, 29], indicating the high potential for the transmission of PPRV through excreted bodily fluids during these peak periods. More importantly, the presence of viral RNA in samples collected before the appearance of any clinical symptoms as highlighted in earlier work [9] emphasizes the critical need for early and proactive sampling in disease monitoring and outbreak management. Detecting PPRV before clinical signs manifest offers significant advantages for controlling spread, particularly in densely populated livestock areas where rapid transmission can lead to large-scale outbreaks.

In cattle, the sporadic RT-qPCR positivity noted throughout the study period indicates a more contained viral presence compared to goats, with the virus tending to localize within specific tissues. This distinct pattern of viral distribution may be explained by species-specific differences in the expression of viral receptors. Previous studies [11, 25, 33] have identified that variations in the expression of PPRV receptors, namely SLAM and nectin-4, across different species and tissues, play a crucial role in determining the susceptibility and resistance to PPRV infection. In cattle, these receptors are expressed differently compared to goats, potentially contributing to the lower and more localized presence of viral RNA found in bovine tissues [33].

The gross and histopathological examinations experimentally infected goats and cattle highlighted the wide range of lesions that result from infection with PPRV, with goats exhibiting a more severe and extensive range of pathology, typical of morbillivirus infections. These findings are consistent with reports from previous studies [14, 27, 31], highlighting the consistent pathological impact of PPRV in caprine hosts. In contrast, cattle inoculated with PPRV/Ethiopia/Habru/2014 presented, nonspecific mild lesions, unlike from a recent studies [35], which noted an absence of observable PPR-related pathology in cattle. These differences highlight variable pathogenic impact of the virus on different host species or might reflect variations in individual or breed-specific immunity.

The use of VDS cells in our study merits further discussion. These cells, derived from the original Vero cell line, are particularly suited for PPRV propagation due to their lack of beta interferon production, which increases their susceptibility to morbilliviruses [15, 37]. While VDS cells offer exceptional virus production capabilities, it's important to note that repeated passages in cell culture can lead to genetic drift and potential attenuation of the virus. Importantly, studies on morbilliviruses, including PPRV, have shown that mutations can accumulate during serial passages in cell culture. For instance, the Rinderpest virus (RPV) isolated after 49 passages resulted in an attenuated strain that did not revert to its pathogenic form[5]. Similarly, the Nigerian strain PPRV Nig 75/1 was attenuated through serial passage in Vero cells, leading to the development of an avirulent vaccine strain [12]. Eloiflin et al. [13] further demonstrated that the PPRV vaccine strain Nigeria 75/1 undergoes genetic changes during cell culture passage, resulting in attenuation [13]. These mutations, while individually may not be fully attenuating, can cumulatively lead to complete attenuation of the virus [20]. In our study, we used the virus at the fourth passage to minimize potential genetic drift. However, we recognize that even this limited number of passages may have introduced some mutations. This consideration is important when interpreting our results, particularly in the context of virus isolation from cattle samples. Future studies could benefit from deep sequencing analysis to track any genetic changes that may occur during cell culture passage.

Importantly, the successful isolation of live PPR virus from bovine samples, though limited, necessitates a reconsideration of the epidemiological role of cattle in the spread of PPRV. The isolation of the virus from a nasal swab at 8 dpi and from select tissues at 6 dpi is particularly revealing. These findings suggest that cattle may potentially act as reservoirs or silent spreaders of the infection under certain conditions, with implications for PPR control and eradication strategies..

Our results both align with and contrast previous studies. Sen et al. [36] reported PPRV detection in cattle blood and PBMCs for extended periods, though not in natural secretions [36]. Conversely, Couacy-Hymann et al. [10] reported that cattle do not exhibit signs of viral replication in epithelial cells and do not excrete the virus in quantities sufficient to infect animals in close proximity [10]. While our study also showed no clinical signs of infection in cattle following PPRV exposure, we did confirm viral excretion through both RT-qPCR and virus culture.

The discrepancy in results could be attributed to several factors, including differences in virus strain, route of infection, virus titer used for inoculation, sampling schedule, and cattle breed. These variables may have contributed to the recovery of live virus from natural secretions (nasal swab at 8 dpi) in our study, unlike in some previous studies.

### Study strengths and limitations

This study offers several strengths, including the use of a local PPRV strain and indigenous breeds, providing regionally relevant data. Our daily postmortem examinations from 1 to 8 dpi offer a detailed temporal analysis of PPRV tissue tropism, which is not commonly reported in such granularity. Additionally, our comparative approach, studying both goats and cattle simultaneously under the same experimental conditions, provides direct insights into species-specific differences in PPRV pathogenesis.

However, some limitations should be noted. Only a single lineage of PPRV (Lineage-IV) was used, limiting generalizability to other strains or regions. The use of a small number of animals, while larger than many previous studies, limits the statistical robustness of the findings. Immunohistochemistry studies were not performed due to lack of access to necessary reagents in Ethiopia, which could have provided additional insights into viral localization and tissue-specific viral dynamics.

Our study did not account for potential confounding factors such as pre-existing infections or exposures, which could influence the observed disease progression and immune responses. Importantly, our findings did not confirm that the virus isolated from cattle was transmitted to susceptible animals, such as goats, through contact. However, we addressed this aspect in another study using the same local virus [19].

Addressing these limitations in future research is essential for a deeper understanding of PPRV pathogenesis and for refining disease control strategies.

### Concluding comments

Taken together, this study presents a comprehensive comparative analysis of PPRV infection dynamics in goats and cattle. The evidence of subclinical infection and live virus shedding in cattle, compared with the moderate clinical manifestations in goats, highlights the complex interaction between PPRV and its hosts. Importantly, the results suggest that, under certain conditions, cattle might play a more active role in PPRV epidemiology than previously understood. The implications of these findings are important for the development of control strategies and diagnostic approaches for PPR in endemic regions. Overall, the findings highlight an urgent need for further experimental studies on cattle PPRV with diverse PPRV lineages in future studies to enhance our understanding of cattle's role in PPRV epidemiology, particularly in regions where the disease is endemic. Additionally, future studies should explore the effects of co-infections and induced stress in goats to better understand how these factors influence the clinical and molecular dynamics of PPRV infection.

#### Abbreviations

| | |
|---|---|
| AHI | Animal Health Institute |
| ALIPB | Aklilu Lemma Institute of Pathobiology |
| ARSERC | Animal Research Scientific and Ethics Review Committee |
| CT | Cycle Threshold |
| dpi | Days post inoculation |
| Ic-ELISA | Immunocapture Enzyme Linked Immuno Sorbent Assay |
| LOESS | Local Regression |
| PPR | Peste des petits Ruminants |
| PPRV | Peste des petits ruminants virus |
| PSU | Pennsylvania State University |
| SLAM | Signaling Lymphocyte Activation Molecule |
| VDS | Vero Dog Slam |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04313-3.

Supplementary Material 1.

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**Authors' contributions**
FA, DSi, and DSh conducted the experiments. FA, AA, RB, MK, DSi, DSh, and AS performed laboratory analyses. FA, CMH, VK and HASh designed the study. CMH created analysis code and plotted figures. FA, CMH, VK, AS and GM analyzed the data. FA, DSi, DSh, and HASg collected samples and data. FA, CMH, and HASg were responsible for data management. FA, DSi, TC, GM, CMH and VK for overall project management. TC and GM project administration. FA wrote the original draft that was extensively revised by CMH and VK. FA, HASh, TK, HC, GM, AS, CMH, CS and VK critically edited and reviewed the manuscript. All authors read and approved the final manuscript.

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**Data availability**
All data and R-code are provided (https://github.com/cherz4/pprv-cattle-goat-comparative-pathology). Whole genome sequence information for PPRV/Ethiopia/Habru/2014 passage 4 can be found at Genbank accession number: ON110960.

## Declarations

**Ethics approval and consent to participate**
The study protocol was ethically and scientifically evaluated by Animal Research Scientific and Ethics Review Committee (ARSERC) of AHI formerly NAHDIC and awarded Ethical Clearance Certificate with certificate Ref. No. ARSERC/EC/001/17/04/2019 on date May 03/2019. In addition, Institutional Review Board (IRB) of Addis Ababa University, Aklilu Lemma Institute of Pathobiology (ALIPB) has awarded Ethical Clearance Certificate with Ref. No. ALIPB IRB/83/2014/22 on date July 21, 2022. This study was reported according to ARRIVE guide lines.

**Consent for publication**
Not Applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's Note