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UDC 619:578.831.2:636.3(574) **Research article**

Persistence of infectious peste des petits ruminants virus in clinical materials and environmental matrices

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Abstract

Background and Aim. Peste des petits ruminants (PPR) is a major transboundary infection causing substantial economic losses in sheep and goat farming. For epizootic surveillance and laboratory diagnostics, data on the persistence of peste des petits ruminants virus (PPRV) in clinical material and environmental matrices are essential, yet remain limited. This study aimed to quantitatively assess the persistence of infectious PPRV in clinical material and selected environmental objects and to determine the effect of key physicochemical factors on the loss of infectivity.

Materials and Methods. Infectious virus was recovered from organs/tissues of deceased animals, excretions/secretions from diseased animals, and residual drinking water after storage under controlled conditions. Virus infectivity was assessed after isolation in a susceptible primary lamb kidney cell culture and quantified by titration ($TCID_{50}$). Stability was evaluated under different temperature regimes, desiccation, extreme pH values, freezing (−10 °C and −50 °C), and lyophilization under vacuum and non-vacuum packaging.

Results. Under isolator conditions (14-16 °C, ~80% relative humidity), infectious virus was generally recoverable from carcass material and secretions mainly within the first 24-48 hours and was not recoverable after 48 hours. Freezing markedly prolonged infectivity: virus-containing material retained infectious activity for 6.5 ± 0.5 months at −10 °C and up to 24 months at −50 °C (observation period). Lyophilized material stored under vacuum at −10 °C remained infectious for at least 18 months with a substantial reduction in titre, whereas non-vacuum storage resulted in loss of infectivity within 3-5 days. In model matrices, infectivity declined rapidly at positive temperatures, during drying, and at extreme pH values; heating to 80 °C/boiling caused immediate inactivation.

Conclusion. PPRV infectivity in clinical material and environmental matrices is strongly determined by temperature, pH, and desiccation, while freezing and properly packaged lyophilization provide prolonged preservation. The results support practical recommendations for sampling, storage, and transport of material for PPR laboratory diagnostics.

Keywords: environmental stability; freezing; infectivity persistence; lyophilization; peste des petits ruminants virus (PPRV).

Introduction

The growth of international trade, seasonal migrations, and cross-border movements of animals substantially increases the risk of introduction and dissemination of zoonotic and anthropozoonotic pathogens, posing a threat to food security and the resilience of agriculture [1]. Among the critically important threats to small ruminants is peste des petits ruminants (PPR), a highly contagious disease of sheep, goats, and several wild ruminants caused by a Morbillivirus of the family Paramyxoviridae (PPRV) [2-5]. Clinically, PPR ranges from mild subclinical forms to severe generalized disease with high mortality in susceptible populations [6, 7, 8]. In response to the economic and epizootic burden of PPR, international organisations such as WOAH (World Organisation for Animal Health) and FAO (Food and Agriculture Organization of the United Nations) have established a disease eradication programme, underscoring the need for applied research aimed at improving epizootic surveillance, diagnostics, and control measures [5, 7, 9, 10].

For practical epizootic surveillance and laboratory diagnostics, data on the persistence of PPRV in the external environment and in clinical material are of particular importance. In the literature, information on PPRV stability in real biological matrices (cadaveric tissues, excretions, water, feed, surfaces) and on the significance of fomite transmission under field conditions remains fragmentary. Available evidence indicates rapid inactivation by heating and desiccation, whereas lyophilized or frozen materials can retain infectivity for substantially longer periods, with direct practical implications for sampling, storage, and the logistics of transporting material to the laboratory [1, 11-15]. Clarifying virus persistence in typical environmental objects and clinical matrices is necessary both for the correct interpretation of laboratory findings and for planning anti-epizootic measures.

Some issues remain the subject of scientific debate. Controversial points include the true epidemiological significance of fomite transmission under typical field conditions [13, 14], as well as the possibility of extrapolating data on the stability of vaccine strains to the behaviour of field isolates [12, 16]. Resolving these controversies requires comparable experimental approaches, studies of virus persistence in representative matrices, and standardized laboratory procedures for determining infectivity.

Until 2014, cases of PPR in Kazakhstan were not officially recorded, and the disease was traditionally considered exotic for the region. In 2014, epizootic outbreaks were described in the Zhambyl, Kyzylorda, and Almaty regions, with a clinical picture typical of PPR [17]; however, these events were not formally reflected in state veterinary reports. Nevertheless, the country as a whole retains the status recognized by WOAH as free from PPR [18]. In a region characterised by intensive agricultural trade links and cross-border movements, the practical importance of data on PPRV persistence is evident: a lack of systematic epizootic surveillance and limited capacity for sample preservation under field conditions may lead to cryptic circulation and delays in response following virus introduction [19-21].

On this basis, the aim of the present study was to quantitatively assess the persistence of infectious PPRV in clinical material and environmental objects, and to determine the influence of key physicochemical factors on the loss of infectivity. The study investigated virus stability in organs and tissues of deceased animals, in excretions and secretions from diseased animals, and in water, as well as the effects of temperature, desiccation, extreme pH values, freezing, and lyophilization on the preservation of infectivity. The resulting data are intended to substantiate practical recommendations for sampling, storage, and transport of material and to improve the effectiveness of epizootic surveillance and laboratory diagnostics for PPR.

Materials and Methods

Ethical approval

All animals used in this study were owned by the Research Institute for Biological Safety Problems (RIBSP), Committee of Science, Ministry of Education and Science of the Republic of Kazakhstan. No client-owned or privately owned animals were involved; therefore, written informed consent from animal owners was not required. All experimental procedures involving animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the European Commission Recommendation 2007/526/EC (18 June 2007), and the WHO Guidelines for Ethics Committees Reviewing Biomedical Research

(Geneva, 2000). The study protocol was reviewed and approved by the Local Committee on Biological Ethics of the Research Institute for Biological Safety Problems (Protocol No. 1 dated 14 July 2023).

Virus: Peste des petits ruminants virus (PPRV) strain Nigeria/76/1 was used in this study and was provided by the Microorganism Collection Laboratory of the LLP "Research Institute for Biological Safety Problems" (Gvardeyskiy, Kazakhstan). The isolate was maintained in the institute's virus collection and, prior to use, was passaged in primary lamb kidney (PLK) cell culture; viral material was aliquoted and stored at −70 °C until the experiments. The specificity of PPRV detection was confirmed by real-time RT-PCR for PPRV RNA using the genesig Standard Kit for PPRV (Primerdesign Ltd, UK), as well as by a virus neutralization test.

Animals, sampling, and storage of specimens for assessment of persistence: To assess the persistence of infectious PPRV, organs and tissues of dead animals (lung, intestine, lymph nodes, spleen), excretions and secretions (nasal secretions, saliva, faeces), and residual drinking water from troughs after use by diseased animals were examined. Material was obtained from infected animals of three groups: Cameroonian goat kids (n=4), local goat kids (n=4), and saiga antelopes (n=4). Sampling was performed immediately after animal death (carcass material) or during clinical disease (excretions/secretions, water).

Samples were stored in the isolator at 14-16 °C and relative humidity of approximately 80% and were tested at 0, 6, 12, 24, and 48 h.

Virus isolation and cell cultures: For virological isolation of PPRV from the tested matrices, primary lamb kidney (PLK) cell culture was used, prepared from animals aged 30-45 days and cultured in PSP medium supplemented with 10% fetal bovine serum (FBS; Gibco, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL). Monolayers were inoculated with the tested material, incubated, and assessed daily for cytopathic effect (CPE). In the absence of CPE, one blind passage was performed in PLK culture. Samples with absent CPE, as well as samples in which the infectious titre could not be determined, were additionally examined by reverse transcription real-time PCR (real-time RT-PCR) using the genesig Standard Kit for PPRV (Primerdesign Ltd, UK). Virus detection was recorded on the basis of recovery after blind passage and/or detection of PPRV RNA by real-time RT-PCR.

After pronounced CPE developed, virus-containing material was harvested after one freeze–thaw cycle, centrifuged at 2,000 × g for 10 min, aliquoted, and stored at -70 °C.

Identification of the isolated virus was performed using RT-PCR (QIAGEN OneStep RT-PCR Kit, QIAGEN, Hilden, Germany) and a virus neutralization test.

Determination of infectious titre: Infectious activity was determined by titration in 96-well plates (8 replicates per dilution) in parallel in PLK, Vero, and chicken fibroblast cultures. $TCID_{50}$ values were calculated by the Reed–Muench method. If infectious activity after primary isolation was insufficient for titre determination, additional passages were performed in PLK culture, followed by titration.

Freezing and lyophilization: In the laboratory, virus stability was studied in tissue suspensions, excretions/secretions, and culture fluid at −10 °C and −50 °C, as well as in lyophilized samples. Lyophilized material was placed into ampoules that were either sealed under vacuum or left unsealed; after storage, infectivity was assessed by virological isolation in PLK cell culture followed by determination of infectious activity.

Effect of temperature, pH, and desiccation: To assess virus stability, three matrices were used: culture fluid containing 5% fetal serum, drinking water (pH 6.8-7.2), and pelleted feed moistened with a virus-containing suspension. Experiments were conducted under four temperature conditions: 4 °C, 18-20 °C, 37 °C, and 80 °C. The effect of pH was evaluated in Hanks' solution at fixed preset pH values of 7.0, 6.1, 3.0, and 8.0 at 18-20 °C. To model desiccation, the virus suspension was placed onto the bottom of a Petri dish and maintained at 18-20 °C until complete drying. The dried residue was subsequently resuspended in culture medium and examined for the presence of infectious virus.

Statistical analysis: Infectious titres are presented as mean ± SD log10 TCID50. Quantitative data, where applicable, were analysed by ANOVA followed by Sidak's multiple comparisons test using GraphPad Prism v10.6.0 (GraphPad Software, USA). A p-value < 0.05 was considered statistically significant. Qualitative virus recovery/non-recovery results were interpreted descriptively.

Results and Discussion

Stability of PPRV in excretions and secretions from diseased animals and in organs and tissues of deceased animals.

Recovery of infectious virus from carcass material, excretions, and secretions – general observations.

When investigating organs and tissues of animals that died of PPR, as well as excretions and secretions from diseased animals, infectious virus could generally be isolated during the first 48 h of material storage in the isolator. Samples were stored under the conditions described in the Materials and Methods section (14-16 °C, ~80% relative humidity). The duration of detectable infectivity correlated with the level of infectious activity: at higher infectious activity the virus remained infectious for a longer period.

Stability in tissues and secretions in different species

In samples from Cameroonian kids, the infectious activity of the virus determined after isolation on PLK was 3.19 ± 0.8-5.08 ± 0.2 $\log_{10}$ $TCID_{50}$/g; infectious virus in organs/tissues and in secretions persisted for 24-48 h. In carcasses and secretions of saiga antelopes, infectious virus could be isolated up to 24 h after death. In cases where infectious activity determined after isolation was low (≤ 1.0 $\log_{10}$ $TCID_{50}$/g; local kids), infectious virus could not be isolated from carcass organs after ≥6 h, whereas in excretions and secretions (samples with activity ~1.87 ± 0.5 $\log_{10}$ $TCID_{50}$/g) it sometimes persisted up to 24 h. Residual water in drinking troughs after use by diseased animals retained infectious virus within 12-24 h, although successful isolation from water was rare.

The results of studies of carcass organs, excretions and secretions from diseased animals, and residual water in drinking troughs after storage in the isolator are presented in Table 1. Table 1 shows that PPRV in these objects was progressively inactivated, and after 48 h infectious virus was not isolated (i.e., it was below the detection limit of the applied virus isolation scheme).

Effect of storage temperature (freezing)

Freezing of samples substantially increased the duration of virus infectivity. Thus, virus-containing material with an infectious activity of 4.08 ± 0.3 $\log_{10}$ $TCID_{50}$/g, when stored at −10 °C, retained infectious activity for an average of 6.5 ± 0.5 months, whereas at −50 °C activity was preserved for up to 24 months (observation period).

Effect of lyophilization and packaging conditions

Lyophilized virus in ampoules with an infectious activity of 4.58 ± 0.5 $\log_{10}$ $TCID_{50}$/g, when stored under vacuum at −10 °C, retained infectivity for at least 18 months, although an average reduction in infectious activity of 3.16 ± 0.3 $\log_{10}$ was noted. When the storage period was extended to 30 months in such ampoules, infectious virus was not isolated. In contrast, storage of the lyophilizate without vacuum led to a sharp loss of activity, and infectious virus was not isolated within 3-5 days.

Virus stability under the influence of various physicochemical factors

The conducted series of experiments demonstrated that physicochemical factors – temperature, medium pH, and desiccation in the presence of air – substantially determine the persistence of cultivable PPRV. The dynamics of loss of infectivity largely depend on the matrix (culture fluid, water, pelleted feed) and storage conditions; in several series, a near log-linear decline in infectious activity was generally observed with increasing temperature and with pH deviations from neutrality. Quantitative results for key experimental series are given below (see Figures 1-3).

Effect of temperature

A culture suspension (initial infectious activity 5.25 ± 0.14 $\log_{10}$ $TCID_{50}$/mL) stored at 4 °C for two months exhibited a decrease in infectious activity of 1.75 ± 0.25 $\log_{10}$ $TCID_{50}$/mL. At room temperature (18-20 °C) the same batch lost a comparable amount of activity over 10-15 days and had become culture-negative by day 35 (Figure 1).

Table 1. Persistence of PPRV in carcasses, excreta, and secretions from infected animals under isolation conditions

Examined material	Presence and titer of the virus over storage period (hours)														
	Cameroon goat					Saiga					Local goat				
	Initial titer	6	12	24	48	Initial titer	6	12	24	48	Initial titer	6	12	24	48
Respiratory organs	4.58±1.1	4.1±0.9	n/i	2.31±0.6	-	2.23±0.7	1.0±0.2	+	-	-	-	-	-	-	-
Digestive organs	5.08±0.2	4.4±0.6	n/i	3.25±0.5	+	1.75±0.5	+	-	-	-	+	-	-	-	-
Lymph nodes	1.75±0.4	1.25±0.2	n/i	-	-	3.31±0.7	2.0±0.5	1.25±0.3	+	-	+	-	-	-	-
Spleen	0.75±0.2	+	n/i	-	-	3.22±0.9	2.5±0.3	1.75±0.4	+	-	-	-	-	-	-
Feces	3.19±0.8	n/i	n/i	1.5±0.4	-	2.71±0.4	n/i	n/i	+	-	1.87±0.5	n/i	n/i	+	-
Nasal mucus and saliva	3.62±0.7	n/i	n/i	+	-	3.46±0.8	n/i	n/i	+	-	1.20±0.3	n/i	n/i	-	-
Drinking water	+	n/i	+	+	-	+	+	-	-	-	n/i	n/i	n/i	n/i	n/i

Note: "+" – qualitative recovery of infectious virus after blind passage in PLK culture (titer not determined);
"–" – no recovery of infectious virus after blind passages;
"n/i" – not investigated.

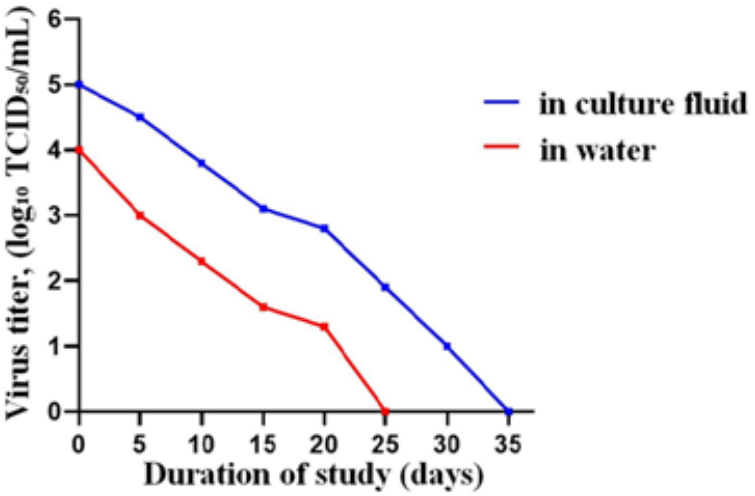

Figure 1. Changes in virus titre at a temperature of 18-20 °C

In the culture suspension maintained at 37 °C, cultivable infectivity was detectable only for 4-5 days (Figure 2).

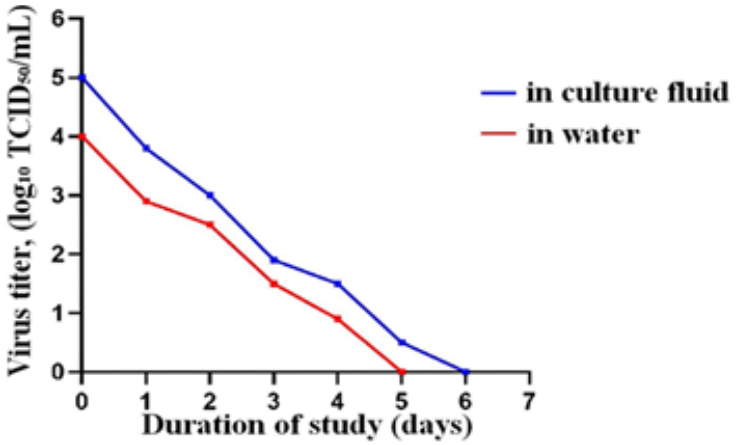

Figure 2. Changes in virus titre at a temperature of 37 °C

Heating to 80 °C and boiling resulted in immediate (culture-detectable) inactivation of the virus.

Matrix: water and pelleted feed

Persistence parameters in drinking water generally corresponded to the results obtained in culture fluid. Water with an initial infectious activity of 4.93 ± 0.40 $\log_{10}$ $TCID_{50}$/mL retained cultivable infectivity up to 25 days at 18-20 °C and 3-5 days at 37 °C. In moistened pelleted feed (initial infectious activity of 2.91 ± 0.50 $\log_{10}$ $TCID_{50}$/g) the virus remained cultivable for approximately 3 days at room temperature and ~1 day at 37 °C. Thus, an organic, adsorptive matrix (feed) accelerates loss of infectivity compared with aqueous media.

Effect of pH and desiccation

pH exerted the most pronounced influence on persistence. In Hanks' solution at neutral pH (7.0 ± 0.05) an initial infectious activity of ≈5.0 ± 0.3 $\log_{10}$ $TCID_{50}$/mL decreased by 0.75 ± 0.20 $\log_{10}$ over 4-5 days at 18-20 °C. At pH ≈6.15 the decline over the same interval amounted to ≈1.91 ± 0.30 $\log_{10}$, whereas at pH ≈3.0 infectious virus was not isolated within 24 h (Figure 3).

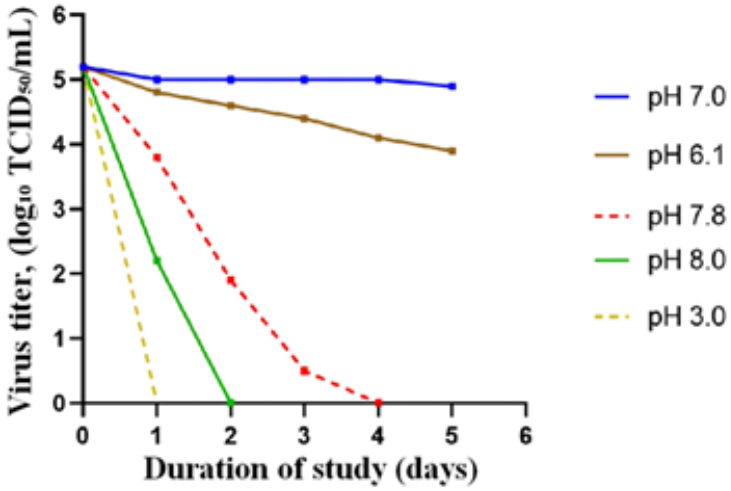

Figure 3. Changes in virus titre at different pH levels of the medium

Alkaline conditions (pH 8.0) led to rapid loss of cultivable infectivity within 1-2 days. The desiccation model showed that in dried droplets of water and culture fluid, infectious virus was not isolated after 24 h; therefore, drying in the presence of air is a critical factor that sharply reduces persistence times.

Stability at subzero temperatures and lyophilization

Frozen material and lyophilized samples stored at low temperatures demonstrated prolonged stability of infectivity. Lyophilization combined with vacuum packaging and storage at low temperatures provides the greatest preservation of the infectious potential of the material.

Interpretation of Results

The persistence of infectious PPRV in clinical material and in the environment appears to be governed by the initial viral load and abiotic factors, primarily temperature, pH, and exposure to drying. Rapid loss of cultivable infectivity at positive temperatures and during desiccation is consistent with the general properties of enveloped viruses, including morbilliviruses, where disruption of the lipid envelope and instability under non-neutral pH conditions contribute to inactivation [22-25]. Conversely, cooling, deep freezing, and lyophilization markedly prolong infectivity, highlighting the critical role of low-temperature storage and appropriate packaging in preserving sample viability for laboratory diagnostics [1, 11, 12, 26, 27, 28].

These results emphasize an important practical distinction: under warm and dry conditions, the potential for fomite-mediated transmission is expected to decrease rapidly over time, whereas in cooled or frozen materials infectious virus may persist substantially longer, particularly when the initial titre is high and the pH remains close to neutral. Benchmarks reported in national guidance documents [29] describing the lability of PPRV outside the host and relative stability within a defined pH range are broadly consistent with the overall patterns observed in our experiments.

At the same time, survival of PPRV under field conditions remains insufficiently characterised. Factors such as matrix composition, fluctuations in temperature and humidity, sunlight/UV exposure, and adsorption to different surfaces may substantially alter persistence. Therefore, further research is required to quantify survival and transmission risks via water, feed, and various surfaces under representative farm conditions, and to refine practical recommendations for sampling, storage, and transport [13, 14].

Conclusions

Infectious PPRV in clinical material and environmental matrices showed limited persistence at positive temperatures and under conditions promoting desiccation. Under isolator storage conditions (14-16 °C, ~80% relative humidity), infectious virus was generally recoverable from organs/tissues, excretions/secretions and residual drinking water mainly within the first 24-48 h, with detectability associated with the level of infectivity.

Freezing markedly prolonged preservation of infectivity in virus-containing material: at −10 °C infectious activity was maintained for an average of 6.5 ± 0.5 months, whereas at −50 °C infectivity was preserved for up to 24 months within the observation period.

Lyophilization and packaging conditions critically affected virus survival. Lyophilized material stored under vacuum at −10 °C retained infectivity for at least 18 months, although a substantial reduction in infectious activity was observed; extension of storage to 30 months resulted in loss of recoverable infectious virus. Storage of lyophilizate without vacuum led to rapid loss of infectivity within 3-5 days.

In controlled model matrices, temperature, pH and desiccation were key determinants of PPRV stability. Heating to 80 °C and boiling caused immediate inactivation; extreme acidic conditions (pH ≈3.0) resulted in loss of recoverable infectivity within 24 h, and alkaline conditions led to rapid inactivation within 1-2 days. Drying in the presence of air eliminated recoverable infectious virus in dried droplets of water and culture fluid after 24 h.

These findings apply to PPRV strain Nigeria/76/1 under the controlled experimental conditions used in this study. Accordingly, the practical implications should be interpreted as guidance for sampling, storage, and transport under comparable conditions rather than as direct estimates of persistence for all field isolates under natural farm conditions.

Author Contributions

Conceptualization, LK; Data curation, BM, and LK; Formal analysis, LK; Methodology, LK and BM; Investigation, GZ, TT, KB, AT, and KZ.; Writing – original draft preparation, LK and BM; Writing – review and editing, BM and LK. All authors have read and agreed to the published version of the manuscript.

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