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Full Length Research Paper

Effect of various physical factors, chemical disinfectants and herbal extracts on the infectivity of Peste des Petits ruminants virus

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ABSTRACT

Peste des petits ruminants (PPR) is a contagious viral disease of small ruminants found in various parts of the world including Pakistan. Present study is focused on to evaluate the effect of various physical factors, chemical disinfectants and herbal extracts on the infectivity of PPR virus (PPRV). The virus as a cell culture harvest was exposed to the physical or chemical agent and residual viral infectivity was measured at regular intervals. Complete inactivation of the virus was achieved at 56°C after 10 minutes of exposure while at 37, 25, and 4°C a drop of 1.00, 1.30, and 2.85 $\log_{10}$ of the virus infectivity was recorded after 2 hours, 24 hours, and 10 days, respectively. After exposing the virus to pH values of 6.00, 7.40 and 8.00 for 2 hours, a drop of 2.00, 1.30, and 2.08 $\log_{10}$ of the virus infectivity was recorded. Similarly the virus was completely inactivated when exposed to ultraviolet radiations for 12 hours. With a starting virus concentration of < 5.00 tissue culture infective $dose_{50}$ ($TCID_{50}$) /ml, a minimum of 0.25% virusnip and 0.001% formaldehyde completely inactivated the virus within 5 minutes and 2 hours respectively while negligible viral infectivity was still detectable after an exposure of 60 minutes to 0.50% pyodine solution. It revealed that herbal extracts were relatively less effective in reducing the virus infectivity. Exposure to mentofin (0.5%), calvangi oil (0.5%) and *Opuntia dellinii* (10%) for 24 hours resulted into the reduction of 3.89, 1.63, and 1.61 $\log_{10}$ virus infectivity.

Keywords: Contagious viral disease, Chemical disinfectants, Herbal extract, pH, PPRV, Temperature, UV light.

INTRODUCTION

Peste des Petits Ruminants Virus (PPRV) belongs to *Paramyxoviridae* and *Morbillivirus*. It is responsible to cause an acute and highly contagious infectious disease of small ruminants (Rashid et al., 2008). PPRV is lymphotropic and epitheliotropic and shows great affinity with the organ systems rich in lymphoid and epithelial tissues (Bailey et al., 2005). The malady is immunosuppressive in nature, having high morbidity and mortality rates of 100 % and 90 %, respectively (Dhar et al., 2002). Currently, the disease is found in various parts of the world specifically in several Africa and Asia countries

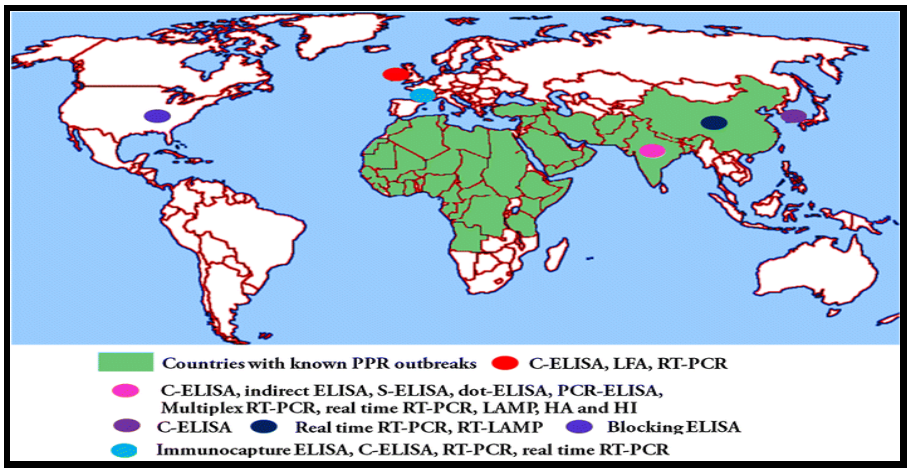

Figure 1. PPR threatened areas of the world
Source: Santhamani , Singh and Njeumi (2016). https://doi.org/10.1007/s00705-016-3009-2

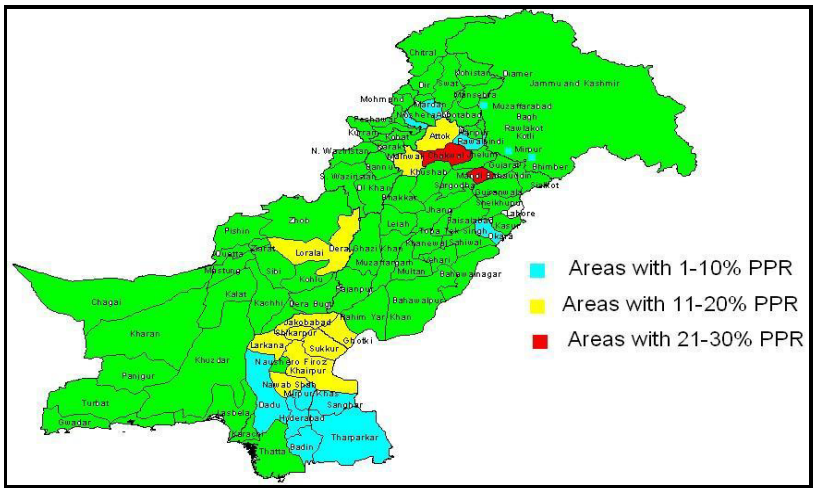

Figure 2. PPR threatened areas of Pakistan

including Pakistan (Figure 1). Several factors are alleged to be responsible for its spatial spread over time in which physical and biological factors have played significant role. It was thought earlier that the problem is restricted to West Africa, but now it has spread out through contagious diffusion to most of the East, West and Central African regions, extending eastward up to West and South Asia (Waret-Szkuta et al., 2008). Due to its harms to ruminants several studies, hitherto, have been conducted on the issue in various parts of the world (e. g. Bamouh, Fakri, Jazouli *et al.* 2019; Mapaco L et al. 2019; Baron MD et al. 2017; Jones BA et al. 2016).

During the last few years, the outbreaks of PPR have reached to alarming levels in Pakistan (Figure 2), involving newer areas (Ali, 2004; Ahmad et al., 2005). The infected animals excrete high amount of virus in their nasal secretion and fecal material which contaminate the environment and responsible for the transmission of the virus to the susceptible population (Khan et al., 2008). In present study, we have find out the survival of PPRV after interaction with common physical factors (temperature, pH and ultraviolet radiations), chemical agents (pyodine, virusnip and formaldehyde) and herbal products (mentofin, black seed oil and Opuntia dellinii extract).

MATERIAL AND METHODS

Cells and Virus

Vero cells were procured from the culture bank of the

Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan. The cells were maintained in disposable vent T-75 cell culture flasks and attained confluent monolayer within 48 hours when fed with growth medium: Dulbecco modified minimum essential medium (DMEM) supplemented with 10% fetal calf serum (FCS) and incubated at 37°C and in 5% CO_2 (Freshney, 2010). Vaccine strain (Nigeria 75/1) of the PPRV was taken from the culture bank of the Department of Microbiology. After cell monolayer was sub-confluent (70-80% confluent), spent medium was removed from the flask and 18-20ml of the maintenance medium (DMEM supplemented with 2% FCS) containing PPRV in a final concentration of 10^3.00 $TCID_{50}$/ml was added. The flasks were incubated at 37°C in 5% CO_2 until appearance of initial cytopathic effects (CPE) within 72 hours. Spent medium was harvested as virus and same amount of the fresh maintenance medium was again added into the flasks which were then re-incubated till the appearance of complete CPE within another 3-4 days. The flasks were subjected to three freeze-thaw cycles, centrifuged at 5000 *xg* for 10 minutes and supernatant was collected as virus. The harvested virus was placed in sterile 50 plastic tubes at -80°C till further use.

Infectivity Assay

Virus infectivity as $TCID_{50}$/ml was measured by titration on Vero cells using 96 well cell culture plates according to the Reed and Muench formula as described by (Swayne et al. 2006).

Experimental Design

The cell culture harvest as a source of PPRV was exposed to various physical and chemical insults. Virus infectivity in the triplicate samples was measured in the beginning and at regular intervals hereafter. Reduction in the virus infectivity as $\log_{10}$ values was measured. Furthermore, the data obtained was used to calculate T-90 values (time required for 90 % reduction in the virus infectivity) using linear regression model. Linear regression which is most commonly used statistical method for predictive analysis has been computed and linear regression model has been developed to make the results more easily understandable. Although, regression analysis is mainly used for three basic purposes, causal analysis, forecasting and effect analysis, and trend forecasting, but in current study, this technique has been used to estimate and substantiate the reduction extent in viral infectivity with factors being studied in a specific time. Regression estimates have been used to describe the data and to explain the relationship between dependent variable virus titer and independent variables time and physical, chemical and herbal agents.

Effect of physical factors on the survival of PPRV

Survival of the PPRV at 56, 37, 25, and 4°C was investigated. For each temperature, 1ml of the PPRV suspension was poured in several sterile tubes of 1.5 ml capacity and kept in either water bath adjusted to 56, 37 and 25°C or refrigerator having a temperature of 4°C. Virus infectivity was measured by taking the tubes after 1, 5, 10 and 20 minutes of exposure at 56°C; 30, 60 and 120 minutes of exposure at 37°C; 3, 6 and 24 hours of exposure at 25°C; and 1, 5 and 10 days of exposure at 4°C.

PPRV suspension taken in 3 sterile small glass beakers was adjusted to three pH conditions (6.00, 7.40, and 8.00) by using 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrochloric acid (HCl) solutions and kept at room temperature (25 ± 3°C). Virus infectivity was measured by taking sample from the suspension after each 30 minutes interval for a total of 2 hours.

In order to investigate the role of UV light to inactivate PPRV, 2 ml of the virus suspension was taken in three 25 mm tissue culture petri plates each and exposed to UV light of wave length 253.7 nm in a class-II safety cabinet at 25 ± 3°C. Samples were taken from the plates at 0, 6, 12, 24 and 48 hours interval to measure residual virus infectivity.

Effect of disinfectants on the survival of PPRV

Three chemical disinfectants: pyodine, virusnip and formalin were tested to evaluate their inactivation potential against PPRV. The virus as cell culture harvest was exposed to 0.25, 0.5, 1 and 2 % working concentration of pyodine and virusnip while 0.1, 0.01 and 0.001 % final concentration of formalin. The suspension was placed at room temperature (25 ± 3°C) and virus infectivity in the suspension was measured at 0, 1, 5, 10, 15, 30 and 60 minutes post exposure (PE) for pyodine and virusnip while at 0, 12, and 24 hours PE for formalin.

Effect of herbal extracts on the survival of PPRV

Inactivation potential of mentofin, black seed oil and Opuntia dellinii extract against PPRV was also

Table 1. Linear regression model showing reduction in the infectivity of PPRV following exposure to various concentrations of chemical disinfectants

Disinfectant concentration	Pyodine		Virusnip	
	Linear regression model	R^2	*Linear regression model*	R^2
0.25%	y= 4.5327 - 0.506x	0.5682	y= 7.8991 - 2.6751x	0.9934
0.5%	y = 4.2519 - 0.523x	0.7458	y= 8.863 - 4.4315x	1.00
1%	C.T	C.T	y= 8.863 - 4.4315x	1.00
2%	C.T	C.T	C.T	C.T

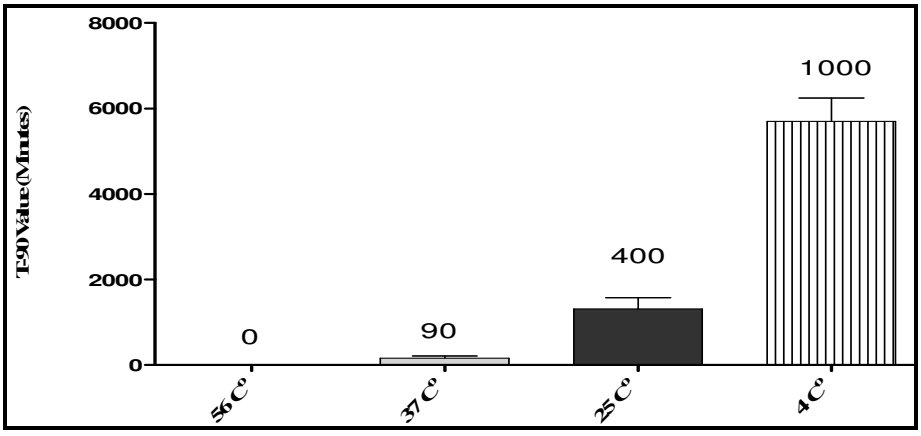

Figure 3. Comparison of T-90 values of the PPRV (95/1 strain) at 56, 37, 25 and 4°C

evaluated. The virus was mixed with 0.01, 0.05, 0.1 and 0.5 % working concentration of mentofin and black seed oil while 1, 5 and 10 % concentration of Opuntia dellinii extract. Residual virus infectivity in the suspension kept at room temperature (25 ± 3°C) was measured at 0, 12 and 24 hours PE.

RESULTS

Effect of Physical factors on the survival of PPRV

In order to compare the effect of temperature on the survival of PPRV, T-90 values at various temperatures were calculated. Figure 3 shows that it needs 2 and 137 minutes at 56 and 37°C while 21 and 941 hours at 25 and 4°C for one $\log_{10}$ reduction in the virus infectivity.

Linear regression model showing reduction in the viral infectivity following exposure to various pH conditions is given in the Figure 4. Survival of the virus was highest at pH 7.40 in comparison to acidic or alkaline conditions. With a mean starting virus concentration of 5.55, 5.35 and 5.43 $TCID_{50}$/ml at 6:00, 7.40, and 8.00 pHs, respectively, an amount of 3.55, 4.05 and 3.35 $TCID_{50}$/ml of the virus was detectable after 2 hours of exposure. UV radiations have drastic effect on the survival of PPRV. Virus suspension with mean infectivity titer of 5.45 $TCID_{50}$/ml was completely inactivated within 12 hours of exposure.

Effect of chemical disinfectants and herbal extracts

Effect of pyodine and virusnip on the infectivity of PPRV is given in Table 1. Following exposure to 0.25 and 0.50% working concentration of pyodine a drop of 3.00 and 3.25 log 10 TCID50 of the virus infectivity was recorded. With a starting virus concentration of 5.25 $TCID_{50}$ /ml, 0.50 and 1% virusnip completely inactivated the virus within 1 minutes while 0.25% virusnip took 5 minutes to inactive the virus. Working concentrations of 0.1 and 0.01% formaline were cytotoxic while exposure to 0.001% completely inactivated the virus within 24 hours.

Effect of mentofin and black seed oil on the infectivity of PPRV is given in Table 2. Following exposure to 0.01, 0.05, 0.1 and 0.5% working concentration of mentofin a drop of 0.15, 1.13, 1.83 and 3.79 log 10 $TCID_{50}$ /ml of the virus infectivity was recorded. With a starting virus concentration of 5.18 $TCID_{50}$ /ml, 0.01 and 0.05% black seed oil reduced the titer to 4.35 $TCID_{50}$ /ml inactivated the virus within 24 minutes while 0.1 and 0.5% black seed oil

Table 2. Linear regression model showing reduction in the infectivity of PPRV following exposure to various concentrations of herbal extracts

Herbal extract concentration	Mentofin		Black seed oil	
	Linear regression model	***R^2***	***Linear regression model***	***R^2***
0.01%	y=5.3482-0.125x	0.75	y=5.5691-0.4157x	0.9865
0.05%	y=5.8276-0.5674x	0.9455	y=5.4857-0.4157x	0.8226
0.1%	y=5.8575-0.9157x	0.8295	y=5.8761-0.7421x	0.9878
0.5%	y=10.671-0.696x	0.9957	y=5.8931-0.8174x	0.9521

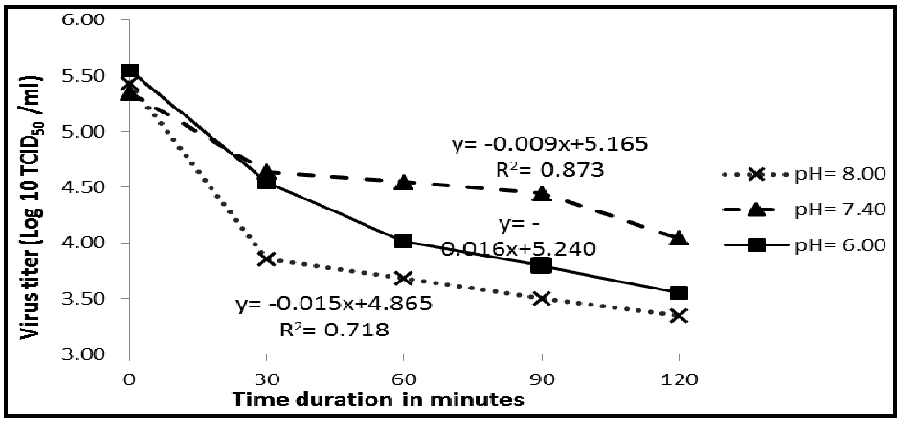

Figure 4. Linear regression model showing decrease in the infectivity of PPRV after exposure to various pH conditions

reduced the titer from 5.18 $TCID_{50}$/ml to 3.70 and 3.55 $TCID_{50}$/ml. While the working concentrations 1, 5 and 10% of freshly prepared *Opuntia dellini* extract reduced the titers from 5.10 $TCID_{50}$ /ml to 4.70, 4.10 and 3.49 $TCID_{50}$ /ml respectively within 24 hours.

DISCUSSION

In present study effect of temperature, pH, and UV radiations was evaluated on the survival of PPRV in the environment. At 56°C the virus was completely inactivated within 10 minutes, while at 37, 25, and 4°C the virus infectivity was still detectable after 2, 24, and 240 hours, respectively (Figure 3). We selected a wide temperature range to have a better insight into the survival of PPRV at diverse environmental conditions. Temperatures of 37°C and 4°C represent hot and cooler months while 25°C for the moderate months of year in Pakistan. Some previous studies documented half-life of PPRV as 24.2 days, 9.9 days, 3.3 hours and 2.2 minutes at -20, 4, 37 and 56°C respectively (Rossiter and Taylor 1994). Being an enveloped virus, PPRV is highly sensitive to temperature and inactivated within 1 hour at 50°C (Lefevre, 1987) as temperature associated inactivation of enveloped virus in comparison to the non-enveloped ones is well documented by Nims and Plavsic (2012). Variation in pH was not found much effective to completely inactivate PPRV within 2 hours but the infectivity titers were reduced at 6.00 and 8.00 as compare to neutral pH (Figure 5). PPRV in diluents with pH 5.85 - 9.5 remains stable. pH below 4.0 or above 11.0 inactivates the virus (Anonymous, 2003). Ultra Violet (UV) light at a wavelength of 253.7 nm is detrimental for PPRV. It inactivated the PPRV within 12 hours. UV rays in natural light inactivate the virus by inducing structural changes in viral proteins and nucleic acid, Wilhelm et al. (2002).

Virusnip inactivated the PPRV within 5 minutes at 0.25% concentration and least toxic for the living cells up to 1% concentration (Table 1), concentration of 2% was found to be cytotoxic. The virusnip at 1:400th dilution inactivated either of the two porcine virus i.e. Classical swine fever virus (CSFV) and PRV, within 30 seconds and reduced infectivity titer from $TCID_{50}$ $10^{5.25}$ and $10^{6.5}$/ml respectively to 0 (Bunpapong et al., 2011). Formaldehyde (37.5%: Merk) at 0.12% effectively inactivated PPRV within few hours but was toxic for the living cells at recommended concentration for disinfection. Only the concentration of 0.001% was evaluated for results and found to completely inactivate the virus in 24

hours. So it can be concluded that in virucidal recommended concentration (2%) it is effective to inactivate the virus within minutes, Anonymous (2008). Pyodine at 0.5% concentration was effective to inactivate the PPRV that reduced infectivity titer from $10^{4.60 \pm 0.11}$ to $10^{1.35 \pm 0.11}$/ml (Table 1) while higher concentrations were found to be cytotoxic. Pyodine is also effective against non-enveloped virus in previous studies (Anonymous, 2008).

Amongst three herbal products, Mentofin inactivated the virus effectively. Mentofin was the most effective to inactivate the virus. At 0.5% concentration the viral titer was reduced from $10^{5.18 \pm 0.11}$/ml to $10^{1.29 \pm 0.82}$/ml $TCID_{50}$ within 24 hours (Table 2). While in previous studies antiviral activity of Mentofin was evaluated against AIV and NDV in-vitro and was found to be effective against both (Barbour et al., 2006; Barbour et al., 2010). The antiviral activity of *Nigella sativa* (Calvangi) is not much prominent in in-vitro studies as compared to in-vivo (Salem and Hossain, 2000) because of its effect is found more as immune-potentiating agent than direct effect on virus (Salem, 2005). Our study on Calvangi oil was also showed not much decrease in viral titer after in-vitro interaction and decrease in titer at 0.5% concentration was from $10^{5.18 \pm 0.11}$/ml to $10^{3.55 \pm 0.20}$/ml in 24 hours (Table 2). The other herbal extract used in study was of a Pakistani cactus variety *Opuntia dellinii*. At 10% dilution of freshly prepared extract the reduction in viral titer was from $10^{5.10 \pm 0.11}$/ml to $10^{3.49 \pm 0.23}$/ml $TCID_{50}$ in 24 hours in-vitro. Pakistani variety *Opuntia dellinii* is effective against influenza virus (Shaukat et al., 2011). We concluded that herbal extracts will be more effective in in-vivo therapy against PPR as compared to its effect on PPRV in-vitro. The regression analysis supports the results further.

CONCLUSION

This experimental study concludes that Peste des petits ruminants (PPR) disease has spread spatially as well as temporally involving new areas over time. This viral disease of small ruminants was found in some parts of the world including Pakistan since long. But through contagious diffusion it has spread out in several new areas due to the interference of several factors**.** Present study, after studying the effect of various physical factors, chemical disinfectants and herbal extracts on the infectivity of PPR virus (PPRV) reached the conclusion that former two factors were more effective and the later one was less effective in controlling PPRV. Results of the study can help to design a program for the decontamination of infected farms and their premises by using formalin and virusnip efficiently which may help the farmers to reduce the period of 'farm rest' after the out-break and limit the spread of PPRV. Use of antiseptic like pyodine is found helpful to treat the oral necrotic tissue and stop the secondary infection. While the herbal extracts are found to be helpful for the treatment of PPR infected animals.

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