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Peste des petits ruminants virus infection potentially facilitates bluetongue virus infection in sheep

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Abstract

Peste des petits ruminants virus (PPRV) and bluetongue virus (BTV) are important viral pathogens affecting small ruminants, with infections frequently occurring in subclinical forms. This study investigated the serological association between PPRV and BTV infections in sheep from Ottapidaram taluk, Thoothukudi district, Tamil Nadu, India. A total of 45 serum samples collected from three sheep flocks during November 2018 were examined using competitive ELISA kits for antibodies against PPRV and BTV. Thirty samples were seropositive for PPRV, of which 23 (76.67%) were also BTV seropositive. Among 15 PPRV-seronegative sheep, 6 (40%) were BTV seropositive. Fisher's exact test demonstrated a statistically significant association between PPRV and BTV seropositivity ($p=0.0227$). The odds ratio was 4.929 (95% CI: 1.322-17.33), while the relative risk was 1.917 (95% CI: 1.113-3.951). These findings indicate an association between PPRV and BTV seropositivity, although causality cannot be established from this study.

Keywords: Peste des petits ruminants virus, bluetongue virus, sheep; seroprevalence, competitive ELISA

1. Introduction

Peste des petits ruminants virus (PPRV) is an important viral pathogen of sheep and goats that can cause respiratory, gastrointestinal, and systemic disease in small ruminants (Kumar *et al.*, 2014). However, PPRV infection may also occur in a subclinical or mild form, particularly in partially immune or less susceptible animals, potentially resulting in alterations in host immune function (Sultana *et al.*, 2022) [7]. Such subclinical infections may not be readily recognized clinically but could influence the susceptibility of affected animals to other infectious agents.

Bluetongue virus (BTV), an orbivirus transmitted primarily by *Culicoides* midges, is widely distributed among ruminants including sheep, goat, cattle and buffalo in India (Subhadra *et al.*, 2023). However, overt disease and clinical signs are prominent in sheep, while other infected ruminants remain disease free. Although BTV infection can cause severe clinical disease in sheep, many infections are often subclinical, depending on the virus serotype, host susceptibility, and immune status (Subhadra *et al.*, 2023). Concurrent infections or underlying immunological disturbances may therefore influence the outcome of BTV infection (Bhanuprakash *et al.*, 2008, Caporale *et al.*, 2014) [1, 3, 6].

The immunomodulatory effects associated with PPRV infection raise the possibility that even subclinical PPRV infection could predispose sheep to infection with other pathogens, including BTV (Bhanuprakash *et al.*, 2008; Brodie *et al.*, 1998) [1, 2]. Detection of BTV infection at a higher frequency among PPRV-infected animals may thus indicate a possible association between the two infections. However, seropositivity or concurrent detection alone cannot establish PPRV as a causal predisposing factor, as factors such as vector exposure, age, management practices, geographic location, vaccination status, and other co-infections may also contribute. Investigating the association between subclinical or clinical PPRV infection and BTV infection could therefore provide insights into potential virus-host interactions and

the epizootology of BTV in sheep populations. In this study, serum samples from sheep obtained during November, 2018 in Ottapidaram taluk of in southern Indian district of Thootukudi in Tamil Nadu, were analysed to study the epizootology of PPRV and BTV infections.

Material and Methods

Samples

45 serum samples were separated from blood obtained from sheep during November, 2018 in three locations, Ottudanpatti (n=17), Kandasampuram, (n=14) and Akkanayakanpatti (n=14), in Ottapidaram taluk of in southern Indian district of Thootukudi in Tamil Nadu and stored in -20°C until further processing.

Competitive ELISA for anti PPRV antibodies and anti BTV antibodies

ID Screen® PPR Competitive ELISA kit and ID Screen® Bluetongue Competitive ELISA kit, manufactured by Innovative Diagnostics, Germany was used for screening of anti PPRV and anti BTV antibodies in the sheep serum samples, respectively, according to the manufacturer's instructions. An OD value of less than 50% of negative sample (S/N%) is considered seropositive for PPRV, while an OD value of less than 40% of negative sample (S/N%) is considered seropositive for BTV.

Statistical Analysis

GraphPad Prism 8.4.3 was used for the statistical analysis. Owing to the small sample size of 45, Fisher's exact test was chosen to determine whether there was a statistically significant association between PPRV seropositivity and BTV seropositivity. Baptista-Pike method was used to determine the exact confidence-interval for the odds ratio of PPRV seropositivity as a risk factor for BTV seropositivity. Koopman asymptotic score method was used to calculate the 95% confidence interval (CI) for estimating the risk ratio/relative risk of BTV seropositivity in PPRV seropositive sheep.

Results

Sample collection

The serum were collected from three sheep flocks in the taluk of Ottapidaram in Thootukudi district during monsoon season in November 2018. The sheep flocks were not vaccinated against BTV or PPRV. Additionally, the flocks included four to five goats which served as a "leader goats" during grazing in pastures. The cohabitation of the goats and sheep is a potential source of PPRV infection to sheep as goats are more susceptible to PPRV infection. Since, all the sheep included in this study were raised in the same taluk of Thoothukudi district, it was assumed that seasonal effects, vector exposure, PPRV and BTV exposure history were equal.

Serological status

In the 45 serum samples, 30 sheep were seropositive for PPRV antibodies and 15 were seronegative for PPRV (Table 1 and Figure 1). Among the PPRV seropositive sheep, 23 were also seropositive for BTV antibodies, while 7 were seronegative for BTV. Among the PPRV seronegative sheep, 6 and 9 sheep were seropositive and seronegative for BTV, respectively. The BTV antibody positive status was 76.67% (23/30) in PPRV antibody positive sheep and 40% (6/15) in PPRV antibody negative sheep.

Table 1: The distribution frequencies of the samples based on their serological status for anti PPRV and anti BTV antibodies. The percentage of each category compared to total number of samples is given in brackets.

Total samples = 45	BTV seropositive	BTV seronegative
PPRV seropositive	23 (51.11%)	7 (15.56%)
PPRV seronegative	6 (13.33%)	9 (20.00%)

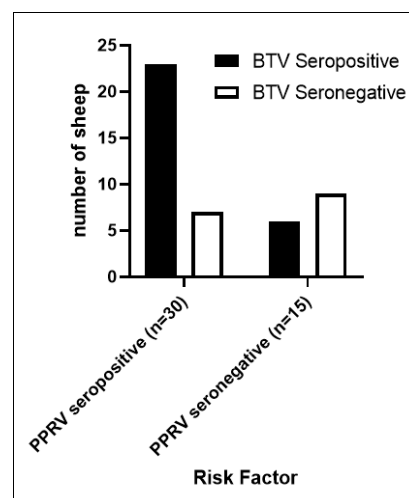


Fig 1: The distribution frequencies of the samples based on their serological status for anti PPRV and anti BTV antibodies.

Statistical tests of association of PPRV seropositivity with BTV seropositivity

Association estimated by two sided Fisher's exact test was $p = 0.0227$ (<0.05) indicated that PPRV seropositivity is significantly associated with BTV seropositivity (Table 2). The odds ratio of a PPRV positive antibody being BTV positive was estimated at 4.929 with at 95% confidence interval of 1.322 to 17.33. The relative risk of PPRV antibody positive sheep being BTV positive was 1.917 with a 95% confidence interval of 1.113 to 3.951.

Table 2: Statistical estimates of association of PPRV seropositivity with BTV seropositivity

Statistical test of association	Estimate	Confidence Interval at 95%
Fisher's exact test	$p = 0.0227$	-
Odds Ratio	4.929	1.322-17.33
Relative Risk	1.917	1.113-3.951

Discussion

The statistical estimates on the association of the PPRV seropositive status with BTV seropositive status indicate a significant association ($p=0.0227$) with an odds ratio of 4.929 and relative risk of 1.917. The PPRV-positive sheep had approximately five times the odds of being BTV seropositivity compared with PPRV-negative animals. However, it should be noted that the sample size of 45 in this study is relatively small, giving a wider estimates at 95% confidence interval. It would be better to investigate the association more robustly with a sample size of more than 200 to achieve higher statistical power. In addition, this analysis evaluates association between seropositivity of PPRV and BTV, not whether PPRV infection predisposes animals to BTV infection. Demonstrating predisposition would require appropriate temporal/clinical evidence, virological parameters and control for confounding factors. It is rather difficult to obtain such data with accuracy from field observations alone and would necessitate a prospective controlled experiment

involving experimental infection with PPRV and BTV in sheep.

PPRV has a strong tropism for small ruminants, which are the principal hosts responsible for maintaining and spreading the disease. PPRV infection, which can occur through out the year in India, can damage lymphoid tissues and cause lymphopenia, leading to immunosuppression (Kumar *et al.*, 2014). It is widely known that PPRV-associated immune dysfunction could potentially facilitate secondary infection and may as well predispose sheep to BTV infection (Brodie *et al.*, 1998; Caporale *et al.*, 2014). The occurrence of BTV in India is associated with the seasonal increase in the vector population in the monsoon season. Subclinical BTV infection generally does not cause marked immunosuppression, but BTV infection may alter host immune responses and, particularly when associated with clinical disease or other stressors, such as PPRV, and could increase susceptibility to secondary infections (Brodie *et al.*, 1998; Caporale *et al.*, 2014). In addition, BTV infection in sheep with certain strains/serotypes can produce lymphopenia/immunosuppression and predispose to secondary infections and potentially exacerbate the clinical condition of an already PPRV infected sheep (Chatzinasiou *et al.*, 2017; Umeshappa *et al.*, 2010) [2, 3, 4, 5, 8].

This study, though with small sample size, indicates a strong association between seropositivity of PPRV and BTV in sheep with equal exposure to PPRV from cohabiting goats, BTV from vectors and seasonal factors in a shared geographical area. This study warrants further detailed investigation with temporal clinical, virological and serological parameters to establish the potential of PPRV infection in sheep as a predisposing factor for BTV infection and influence on clinical outcome.

Conflict of Interest

The authors declare no conflict of interest commercial or otherwise

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