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Comparison of milk ELISA with serum ELISA for detecting the sero-prevalence of infectious bovine rhinotracheitis in cattle

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Abstract
The present study was carried out to study the comparison of milk samples with serum samples for detecting the prevalence of Infectious Bovine Rhinotracheitis using I-ELISA among cattle. A total of 46 serum and 46 milk samples were subjected for the presence of Infectious Bovine Rhinotracheitis (IBR) antibodies using indirect ELISA method and the sensitivity of milk I-ELISA was analyzed as 41.66% and a specificity of 68.18% was observed, the positive predicted value recorded was 58.82% and the negative predicted value of 78.94% was noted, the overall performance of the test was 54.34% and the overall prevalence rate of 52.17% was recorded.

Keywords: I-ELISA, serum, milk, cattle, IBR

Introduction
Infectious Bovine Rhinotracheitis is caused by the bovine herpesvirus type I, belonging to the family Herpesviridae. It is an enzootic disease on the B list of the Office International des Epizooties (OIE, 2010) [6]. In India, the distribution of the disease is of spread wide, Nandi *et al.* (2009) [4] has been extensively reviewed the endemic prevalence of IBR in India. It is one of the most prevalent respiratory and reproductive viral diseases of cattle in India. Kiran *et al.* (2005) [2].
ELISA is a rapid, inexpensive and highly specific test for detecting Ab titre in sera and milk of animals; that is why it is of paramount significance to detect latent virus carriers in control programme, in International trade act, for sero epidemiological studies, sero-surveillance during eradication programme and to evaluate antibody response during vaccination studies. Sharma, *et al*., 2009 [8]. Enzyme Linked immuno Sorbent Assay technique gradually replaced Viral Neutralization test. Several ELISAs are utilized for the detection of antibody in serum samples, however, Kramps *et al*. (2004) [3] detected antibody to BHV1-IBR in milk.
Indirect ELISAs are the most sensitive tests used in the detection of BHV-1 antibodies in milk (OIE, 2010) [6].

Materials and Method
Collection of samples
A total of 46 Milk and 46 Serum samples from animals were selected randomly and the samples were stored at -20 °C before subjecting to serum and Milk ELISA.

Indirect enzyme linked immuno sorbent assay (I-ELISA)
ELISA kit-Infectious bovine rhinotracheitis antibody test SVANOVIR IBR-Ab-10-2100-50Box 1545, SE-751(Fig 1) purchased from Uppsala-Sweden was used for the screening of antibody in milk and serum samples, where ready to use antigen coated (Non-Infectious-IBR) solid phase indirect ELISA system, any serum samples that have IBR antibody would bind to the antigen in the solid phase and the further addition of HRP conjugate subsequently binds with IBR antibodies in the reaction. In addition of substrate it removes the unbounded material and develops blue color in the presence of conjugate and indicates the positivity of the samples. When the reaction is stopped by the addition of stop solution may change the blue color into yellow. Based on the intensity of the developed colour the OD value of the test may differ while measured using a micro plate photometer at 450nm.

Fig 1: Svanovir i-ELISA antibody kit

$$\% \text{ Positivity} = \frac{\text{Test sample or Neg C}}{\text{Positive Control}} \times 100$$

Criteria for test validity

To ensure validity, the duplicate OD values of the positive control should not differ more than 25% from the mean value of the two duplicates.

Additionally, the control values should fall within the following limits:

OD Positive control >0.9

PP Negative Control <15.

Interpretation of test sample results

Serum >18 -Positive
<18 -Negative

Milk <8 - Negative
>- Positive

ELISA procedure for serum samples

10ul serum samples procedure

1. All reagents were equilibrated to room temperature before use.
2. Plates were labelled
3. Serum samples, positive, negative and test serum samples 10ul added to the respective wells in the Pre-IBR antigen coated plate.
4. All the serum samples including positive and negative controls were ran in duplicates for confirmation.
5. Plate was gently rocked side by side and covered with aluminium foil and incubated at 37ºCfor 1 hour or overnight at 2-8ºC.
6. The plate was rinsed three times with 1X PBS -Tween buffer. Wash buffer was completely removed by tapping against the tissue paper.
7. HRP conjugate 100ul was added and the plate was sealed with aluminium foil and incubated as in step 5.
8. After incubation for an hour step 6 was followed.
9. Substrate solution 100 ul to each well was added and incubated for 10 minutes at room temperature. This reaction was indicated by development of blue color.
10. The reaction was stopped by adding 50ul stop solution to each well and gently tapped to mix. The blue color developed in the previous step replaced by yellow color.
11. The optical density (OD) of the controls and samples was measured at 450nm using microplate photometer.
12. The OD was measured within 10-15 minutes after adding stop solution to prevent fluctuation in OD values.

Milk ELISA procedure

1. PBST containing 0.5% skim milk powder 100ul was added to the IBR antigen coated 96 well plate.
2. To which, 10 ul positive and negative control serum was added as controls. Test milk samples 10 ul was added to all test milk sample wells.
3. From now on the procedure used in serum samples testing - From Step 4 to step 12 was used.

Statistical analysis

The data used in this study was subjected to statistical analysis as per the procedure described by Snedecor and Cochran (1994) [9]. The sensitivity and specificity for serum and milk samples were tested by I-ELISA and was analyzed as per the description of Ronald Smith (1994) [7] and is described below in Table 1.

Table 1: Analysis of sensitivity and specificity for serum and milk samples

Screening test result	Disease status		Total
	Present	Absent	
Positive	a (True Positive/TP)	a (False Positive/FP)	(a+b)
Negative	c (False negative/FN)	d (True Negative/TN)	(c+d)
Total	(a+c)	(b+d)	n or (a+b+c+d)

$$\text{Sensitivity} = \frac{a}{(a+c)} \times 100; TP/(TP+FN)$$

$$\text{Specificity} = \frac{d}{(b+d)} \times 100; TN/(TN+FP)$$

$$\text{Positive Predictive Value} = \frac{a}{(a+b)} \times 100; TP/(TP+FP)$$

$$\text{Negative Predictive Value} = \frac{d}{(c+d)} \times 100; TN/(TN+FN)$$

$$\text{Accuracy} = \frac{(a+d)}{(a+b+c+d)}; TP+TN/n$$

$$\text{Prevalence} = \frac{(a+c)}{(a+b+c+d)}; TP+FN/n$$

Result

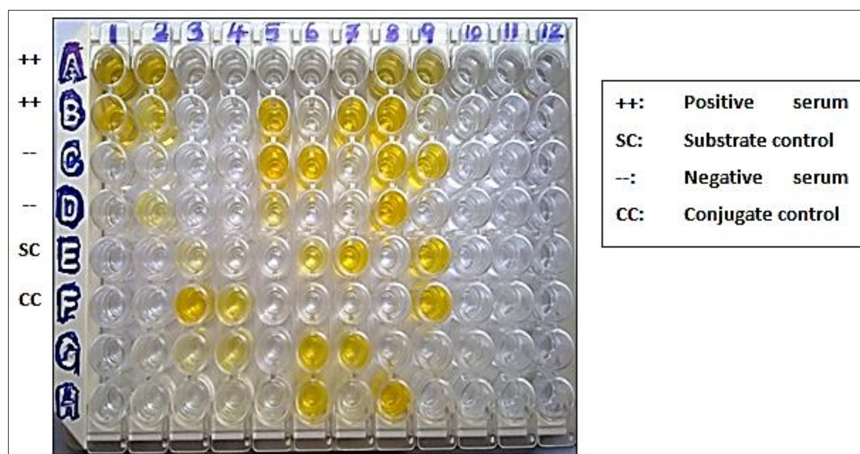

Fig 2: Show the positive Negative serum and substrate conjugate control

Table 2: Serum Elisa

Test		Serum Elisa		Total
		Positive	Negative	
ELISA Milk	Positive	10	7	17
	Negative	14	15	29
Total		24	22	46

Sensitivity = 41.66%
Specificity = 68.18%
Positive Predictive Value = 58.82%
Negative Predictive Value = 78.94%
Accuracy = 54.34%
Prevalence = 52.17%

Discussion

A total of 46 Milk and 46 Serum samples were tested for the presence of IBR antibodies using indirect ELISA method (Fig 2). Out of 46 samples, 10 samples were positive by both milk and serum I-ELISA and 15 samples were negative by both milk and serum I-ELISA. 7 samples were positive by milk I-ELISA and negative by serum I-ELISA, likewise 14 samples tested as positive by serum I-ELISA and negative by milk I-ELISA. As seen in Table 2, overall; 24 samples were positive and 22 samples were negative by Serum I-ELISA and 17 samples were positive and 29 samples were negative by milk I-ELISA respectively. The sensitivity of milk I-ELISA was analyzed as 41.66 per cent and the specificity was 68.18 per cent was observed. Beer *et al.,* (2003) [1], suggested Indirect ELISA was highly sensitive and specific and the results of this type of ELISA can be comparable with gB ELISA. The second generation indirect ELISAs were found to be the most sensitive tests for the detection of IBR specific antibodies in milk (OIE, 2010) [6]. According to Wellenberg *et al.* (1998) [10], gE blocking ELISA might be useful to detect antibody prevalence in herd with 10 to 15 per cent infection, however, it maybe not be sufficient to declare the herd as free of infection and because of this further gE or gB based individual serum based ELISA is warranted. Nylin *et al.* (2000) [5] reported bulk milk can be used to study the prevalence in a herd in an area or in a country, the positive predicted value recorded was 58.82% and the negative predicted value of 78.94% was noted, the accuracy rate or overall performance of the test was 54.34% and the overall Prevalence rate of 52.17%was recorded.

Conclusions

The overall antibody prevalence of milk and serum against Infectious Bovine rhinotracheitis (IBR) by I- ELISA method was observed at 52.17% per cent, the accuracy rate or overall performance of the test was noted at 54.34%. There was no significant difference between the detection and the prevalence of the antibody in milk and serum samples against Infectious Bovine rhinotracheitis by I-ELISA. In this study, milk and serum samples were identified as good source of parallel samples and both can also be used for evaluating the presence of antibody in milk and serum samples in the same animal by I-ELISA method.

Competing interests

The authors declare that they have no competing interests.

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