Record of porcine brucellosis in India by indigenously developed indirect ELISA

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All experimental were conducted in accordance to Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) and approved by Veterinary College, Karnataka Veterinary Animal and Fisheries Sciences, Bangalore (CPCSEA Reg No: 493/CPCSEA dated 31.01.2001).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

\section{1. Introduction}

Porcine brucellosis is a contagious and emerging zoonosis but neglected in most of the endemic countries including India. The disease in pigs is rarely reported due to non-availability of diagnostics or major focus is on bovine brucellosis. Hence, the necessity was felt to develop indirect ELISA for the detection of anti-\textit{Brucella} antibodies and to record spatial seroprevalence of porcine brucellosis in the country. The relative diagnostic sensitivity and specificity of the developed indirect ELISA were 94.0\% and 92.0\%, respectively and kappa agreement with rose bengal plate test, serum agglutination test and commercial indirect ELISA kit was found to be 0.86 (95\% confidence interval 0.78–0.93). A total of 2,576 random serum samples sourced from 10 states were screened by indirect ELISA and true prevalence of 7.2\% (95\% confidence interval 5.6–8.7) was recorded. The study concluded the prevalence of brucellosis in swine population in many states of the country and indirect ELISA as an alternate test to rose bengal plate test and serum agglutination tests.

\section{2. Materials and methods}

\subsection{2.1. Selection of positive and negative serum panels}

Five hundred pig serum samples were collected from pig farms having no previous history of brucellosis and tested negative for anti-\textit{Brucella} antibodies by RBPT, serum agglutination test (SAT) (colored and plain antigens procured from Institute of Animal Health and Veterinary Biologicals, Bangalore, India) and indirect ELISA (Bionote, Gyeonggi-do, Korea). Similarly, 500 serum samples were collected from farms with clinical history of abortions, confirmed by isolation of \textit{Brucella suis} from 5 aborted samples and positive status of sera samples by RBPT, SAT titre > 1:80 and indirect ELISA[11].
2.2. Standardization of in house indirect ELISA

In the first stage of test development, smooth lipopolysaccharide (sLPS) antigen was extracted from *Brucella abortus* S99 as per The World Organisation for Animal Health (OIE) protocol[1] (*Brucella abortus* S99 strain was procured from National *Brucella* Culture Repository, Indian Veterinary Research Institute, Iznagar, Bareilly-243 122, India). In second stage, hyperimmune sera was raised against sLPS antigen in two 8 months old large white Yorkshire male pigs as per standard procedure. The pigs were selected from the herds free of brucellosis and animal ethics committee approval for raising antisera has been obtained from Veterinary College, Hebbal, Bangalore, India. After 5 weeks of immunization, hyperimmune sera was tested for agglutination by RBPT and antibody titre by SAT[1] and analytical sensitivity by end-point dilution method in indirect ELISA (from 1:100 to 1:819200). ELISA protocol was standardized by checkerboard titration method as per Wright et al.[12] using rabbit anti-swine immunoglobulin G-horse radish peroxidase conjugate (Sigma, Missouri, USA) and positive percent positivity cut-off was arrived in comparison to RBPT, SAT titre and indirect ELISA kit (Bionote, Gyeonggi-do, Korea).

To rule out the cross-reactivity of the sLPS antigen used in the assay, *Escherichia coli* (O157 H7), *Salmonella*, n-17 (VI; polyvalent O; polyvalent O1; O1,3,19; O2; O3,10; O4; O6,14; O7; O8; O9; O9,46; O11; O13; O16; O18; O35; O21) and *Yersinia enterocolitica*, n-5 (O1 and 2; O3; O5; O8; O9) serotype specific reference sera (Denka Seiken Co, Tokyo, Japan) were tested. Similarly, OIE international and national (Indian Veterinary Research Institute) reference positive and negative serum samples have also been tested to evaluate the assay performance. The relative diagnostic sensitivity and specificity of in house indirect ELISA were calculated as described by Thrusfield[13] and kappa statistics for the measurement of agreement with RBPT, SAT and commercial indirect ELISA kit.

2.3. Seroscreening of porcine brucellosis using standardized indirect ELISA

A total of 2576 serum samples collected by multi stage random sampling approach from 10 different states were screened by standardized indirect ELISA. All the analysis were carried out using statistical software SPSS version 22 (IBM, New York, India) and true prevalence estimation by using Epi tools (http://epitools.ausvet.com.au) where diagnostic sensitivity, specificity and sample size were taken into consideration[14].

### Table 1

| Status       | Positive | Negative | Total |
|--------------|----------|----------|-------|
| Positive     | 470      | 40       | 510   |
| Negative     | 30       | 460      | 490   |
| Total        | 500      | 500      | 1000  |

### Figure 1

Figure 1. State wise seroprevalence of brucellosis in swine population of India.

4. Discussion

In India, brucellosis in swine is mainly diagnosed by conventional RBPT and SAT tests. These tests are less sensitive, as they fail to detect very low levels of antibodies and in SAT, specificity is reduced by nonspecific antibody thought to be immunoglobulin M[1,15]. Improved efficacy of enzyme based assays in comparison to other tests for diagnosing brucellosis in humans[16], cattle and buffaloes[17] and goats[18] are reported. The present study aimed to standardize indirect ELISA for surveillance of porcine brucellosis in the country. Till date sLPS antigen is proved superior to all other Brucella antigens evaluated[19] and hence sLPS antigen was used for the assay. The sLPS antigen extraction, purification and indirect ELISA procedures were carried out as per standard OIE protocols[16,20]. Brucella antigens share structural similarities with lipopolysaccharide regions of various Gram-negative bacteria, namely, *Salmonella*, *Yersinia*, and *Vibrio*. To rule out cross reactivity, a panel of 23 serotype specific reference sera (*Escherichia coli*, *Salmonella* and *Yersinia*) were evaluated and all the reference sera showed the percent positivity values less than 50 which is...
determined as negative diagnostic cut off percent positivity for the assay. The standardized assay showed specificity and sensitivity of 92.0% and 94.00%, respectively along with 92.16% and 93.88% of positive predictive value and negative predictive value, respectively.

So far, seroprevalence ranging from the lowest 3.2% from Madhya Pradesh[18] to 6.3% and 9.5% in Karnataka[21], to 11.3% in Tamil Nadu[21], 16.7% in Uttar Pradesh[22] to the highest prevalence of 87.10% in pigs with history of abortion from Assam[23] have been reported. In the present study, seroprevalence of 9.9% and 8.5% from Punjab and Karnataka states, respectively are being similarly reported as in earlier reports indicating continued prevalence of the disease in swine herds of these states. Comparatively low seroprevalence of brucellosis in few states (Meghalaya, Rajasthan and Gujarat) should not be ignored because free trade between states facilitates transmission of the disease to low prevalent areas within no time.

The study confirmed brucellosis in few states and further studies in other states of the country is essentially required to map the disease in the country. The standardized indirect ELISA can serve the need to low prevalent areas within no time.

This work is supported by the Indian Council of Agricultural Research through All India Coordinated Research Project (AICRP) on Animal Disease Monitoring and Surveillance. We gratefully acknowledge all the PI and Co-PIs of the AICRP units for contributing samples and Dr. M.R. Gajendragad and Dr. D. Hemadri, Principal Scientists for facilitating the collection of samples. Laboratory help rendered by Mr. Manu Kumar and Mr. B. Hanumantharaju are highly acknowledged.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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