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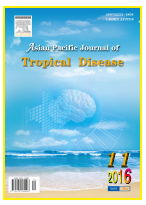

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# Record of porcine brucellosis in India by indigenously developed indirect ELISA

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ABSTRACT

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-*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.

## 1. Introduction

Porcine brucellosis is a contagious disease with greater zoonotic potential characterized by infertility, abortion and birth of dead or weak piglets in sows, orchitis and infection of secondary sex organs in boars and lameness and paralysis in both sexes[1-3]. The disease is generally transmitted during copulation and by consumption of feed contaminated by birth and/or abortion products and uterine discharges[4,5]. The ingrain of infection mainly occurs in organized swine farms where animals from different areas are procured indiscriminately for breeding or fattening purpose without proper disease checks or quarantine. Hence routine screening at the event of every reproductive failure or before introduction of new animals into the farm is very important.

Confirmative diagnosis of brucellosis requires isolation of the causal agent but isolation is highly hazardous[6]. PCR-based assay is not suitable for routine diagnosis[7], rose bengal plate test (RBPT) is compounded with false positive results[8,9] and complement fixation test is not considered suitable, as swine complement interacts with guinea pig complement[1]. The primary binding assays for detection of anti-*Brucella* antibodies have been standardized elsewhere[3,10] and needs to be imported to the country. Hence, present prospective study is aimed to standardize indirect ELISA to facilitate documentation of spatial prevalence of porcine brucellosis in India.

## 2. Materials and methods

### 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-*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 *Brucella suis* from 5 aborted samples and positive status of sera samples by RBPT, SAT titre > 1:80 and indirect ELISA[11].

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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).

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### 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, Izatnagar, 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 entericolitica*, *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].

## 3. Results

The kappa agreement for in house indirect ELISA in comparison to RBPT, SAT and commercial ELISA was found to be 0.86 [95% confidence interval 0.78–0.93] and the sensitivity and specificity at 50% cut off percent positivity were found to be 94.0% and 92.0%, respectively (Table1). Similarly, good positive (92.16%) and negative predictive values (93.88%), respectively were recorded.

Among 2576 random serum samples screened from 10 states, 365 were detected positive by indirect ELISA with apparent prevalence of 14.2 (95% confidence interval 12.9–15.6) and true prevalence of 7.2 (95% confidence interval 5.6–8.7). When the samples were compared state wise, the highest seroprevalence was recorded in samples from Andhra Pradesh (28.2%), Madhya Pradesh (14.6%), Punjab (9.9%) and Karnataka (8.5%) states. The lowest seroprevalence was recorded in samples from Gujarat, Rajasthan and absence of anti-*Brucella* antibody in Meghalaya.

**Table 1**

Contingency table showing results of in house indirect ELISA versus that of RBPT, SAT (> 1:80) and commercial indirect ELISA kit for detection of antibodies in swine.

| In house indirect ELISA | RBPT, SAT and commercial ELISA kit | | | |
|---|---|---|---|---|
| | Status | Positive | Negative | Total |
| | Positive | 470 | 40 | 510 |
| | Negative | 30 | 460 | 490 |
| | Total | 500 | 500 | 1000 |

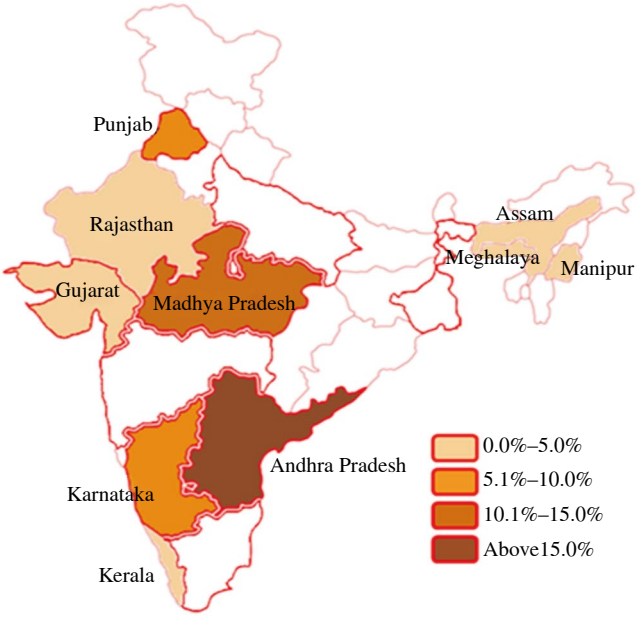


**Figure 1.** State wise seroprevalence of brucellosis in swine population of India.

## 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 for, 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 for brucellosis surveillance in the country which owns 13.51 million swine population. *Brucella* species causes severe infection in human beings and there are reports of *Brucella suis* infecting non-specific host like cattle, buffaloes and wild animals[24]. In our study, a pig farmer and two male handlers were tested brucellosis positive by RBPT, SAT, PCR and human IgG ELISA (unpublished data). Vaccination against swine brucellosis is not practiced in the country and hence continued surveillance and removal of infected pigs should be strictly adopted to control the disease in swine herds.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] World Organisation for Animal Health. Porcine brucellosis. In: *Manual of diagnostic tests and vaccines for terrestrial animals*. Paris: World Organisation for Animal Health; 2009, p. 1106-12.

[2] Woldemeskel M. Zoonosis due to *Brucella suis* with special reference to infection in dogs (carnivores): a brief review. *Open J Vet Med* 2013; **3**: 213-16.

[3] Thirlwall RE, Commander NJ, Brew SD, CutlerSJ, Mc Given JA, Stack JA. Improving the specificity of immunodiagnosis for porcine brucellosis. *Vet Res Commun* 2008; **32**: 209-13.

[4] Shimshony A. Porcine brucellosis: another common zoonosis in pigs. *Zoonotic Infect Infect Dis News* 2009; **22**: 14.

[5] Iowa State University. Porcine and Rangiferine Brucellosis: *Brucella suis*, enzootic abortion, contagious abortion, undulant fever. Ames: Iowa State University; 2009. [Online] Available from: http://www.cfsph.iastate.edu/Factsheets/pdfs/brucellosis_suis.pdf [Accessed on 25 April, 2016]

[6] Ilhan Z, Aksakal A, Ekin IH, Gülhan T, Solmaz H, Erdenlig S. Comparison of culture and PCR for the detection of *Brucella melitensis* in blood and lymphoid tissues of serologically positive and negative slaughtered sheep. *Lett Appl Microbiol* 2008; **6**: 301-6.

[7] Yu WL, Nielson K. Review of detection of *Brucella* spp. by polymerase chain reaction. *Croat Med J* 2010; **51**: 306-13.

[8] Nielsen K, Yu WL. Serological diagnosis of brucellosis. *Prilozi* 2010; **31**: 65-89.

[9] Poester FP, Samartino LM, Santos RL. Pathogenesis and pathobiology of brucellosis in livestock. *Rev Sci Tech* 2013; **32**: 105-15.

[10] Abdoel T, Dias R, Cardoso IT, Smits HL. Simple and rapid field tests for brucellosis in livestock. *Vet Microbiol* 2008; **25**: 312-19.

[11] Nagalingam M, Shome R, Balamurugan V, Shome BR, Narayanarao K, Vivekananda, et al. Molecular typing of *Brucella* species isolates from livestock and human. *Trop Anim Health Prod* 2012; **44**: 5-9.

[12] Wright PF, Nielsen E, Van Rooij EMA, Lelenta M, Jeggo MH. Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. *Rev Sci Tech* 1993; **12**: 435-50.

[13] Thrusfield M. *Veterinary epidemiology*. London: Butterworth; 1986, p. 280.

[14] Rogan WJ, Gladen B. Estimating prevalence from the results of a screening test. *Am J Epidemiol* 1978; **107**: 71-6.

[15] Kaltungo BY, Saidu SNA, Sackey AKB, Kazeem HM. A review on diagnostic techniques for brucellosis. *Afr J Biotech* 2014; **13**: 1-10.

[16] Al Dahouk S, Sprague LD, Neubauer H. New developments in the diagnostic procedures for zoonotic brucellosis in humans. *Rev Sci Tech* 2013; **32**: 177-88.

[17] Shome R, Deivanai M, Shome BR, Narayan Rao K, Prabhudas K. Serological and PCR confirmation of *Brucella suis* abortion in swine. *Indian Vet J* 2011; **88**: 17-9.

[18] Nielsen K, Gall D, Smith P, Vigliocco A, Perez B, Samartino L, et al. Validation of the fluorescence polarization assay as a serological test for the presumptive diagnosis of porcine brucellosis. *Vet Microbiol* 1999; **68**: 245-53.

[19] Gall D, Nielsen K. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Rev Sci Tech* 2004; **23**: 989-1002.

[20] Shome R, Padmashree BS, Krithiga N, Triveni K, Sahay S, Shome BR, et al. Bovine brucellosis in organized farms of India-an assessment of diagnostic assays and risk factors. *Adv Anim Vet Sci* 2014; **2**: 557-64.

[21] Boral R, Singh M, Singh DK. Status and strategies for control of brucellosis-a review. *Indian J Anim Sci* 2009; **79**: 1191-99.

[22] Saini SS, Joshi VB, Khehra R, Ramneek S,Kwatra MS. Swine brucellosis among breeding pigs of Punjab and market pigs of Himachal Pradesh. *Indian J Anim Sci* 1994; **64**: 1177-79.

[23] Nath AJ, Barman NN, Sarma DK, Das SK, Bora DP. Seroprevalence of brucella and parvovirus infections in pigs. *Indian Vet J* 2009; **86**: 339-41.

[24] Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. *Croat Med J* 2010; **51**: 296-305.