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# Cross-species transmission of *Brucella abortus* in an aborted sow

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**Abstract**
Swine brucellosis causes economic losses due to abortion at any stage of gestation in sows; orchitis and epididymitis in boars; sterility and hygroma in adult pigs all over the world. Typically, the infection in pigs is caused by *B. suis* biovars 1–3. Despite the host specificity reported in many species of *Brucella*, there may be cross-species transmission due to breaches in the animal host boundary at mixed or integrated farming systems or at the livestock-wildlife interface. In the study, a case of late abortion in a four year old non-descript (desi) sow was reported at Department of Veterinary Preventive Medicine, Madras Veterinary College, Chennai for screening of brucellosis. *Brucella* antibody was detected from the sow using serological tests like RBPT and indirect ELISA. *B. abortus* biovar 1 was isolated and identified based on bacteriological evaluation and was further confirmed by AMOS PCR. This article depicts the detection of a spillover infection of *B. abortus* biovar 1 in an aborted non-descript sow reared in a small-scale holding. Hence, it is evident that the cross species transmission of *B. abortus* occurred irrespective of its host specificity, highlighting the possibility of novel transmission dynamics within the pigs by direct or indirect contact with infected cattle. This study warrants the need for further investigation of the epidemiology, transmission dynamics and pathogenicity of the zoonotic bacteria.

**Keywords:** Abortion, *B. abortus*, brucellosis, cross-species transmission, pigs

## Introduction
Brucellosis is an anthropozoonotic re-emerging disease with ubiquitous disposition, multifaceted epidemiology and socio-economic implications worldwide [1]. *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis* and *B. canis* are the classical species, preferentially affecting cattle, small ruminants, swine and dogs respectively, that are having a great impact on domestic livestock productivity and human health [2]. Swine brucellosis is characterised by septicaemia, chronic inflammatory lesions in the reproductive organs and joints causing economic losses due to abortion at any stage of gestation, still birth, birth of weak piglets, orchitis, epididymitis, sterility, hygroma, spondylitis especially of the lumbar and sacral regions, with occasional paralysis of the hind limbs and rarely arthritis in swine [3, 4]. Epidemiological data on brucellosis in ruminants are easily available, however, reports on swine brucellosis remain limited [5]. The barriers to cross-species infection are also poorly understood [6], however, there were instances of crossing of animal host boundary have been reported previously viz. *B. suis* and *B. melitensis* in cattle [7, 8], *B. abortus* in swine [9].
Scare information is available about seroprevalence and status of swine brucellosis compared to ruminants due to the lack of gold standard test and problems associated with the specificity of serological tests in porcine species [10, 11]. Laboratory diagnosis of brucellosis should rely either on a positive culture, or PCR result from blood, organ samples or positive IgM and IgG ELISA results [12]. This article gives a limelight about the possibility of vulnerability of swine to *B. abortus* due to the widespread distribution of brucellosis within livestock populations.

## Materials and Methods
A case of late abortion in a four year old non-descript (desi) sow was reported at the Department of Veterinary Preventive Medicine, Madras Veterinary College, Chennai for the screening of brucellosis. The sow was reared under a small-scale holding for pigs wherein the pigs were allowed to range freely scavenging garbage and discarded animal offal. With proper restraint, 2 ml blood sample was collected in clot activator and EDTA vials for serology and bacteriological studies respectively. The vaginal swab, aborted fetal membranes and fetal

stomach contents were collected aseptically and transported to the laboratory on ice within 2 hours of collection for isolation and identification.

**Serological evaluation**

Serum was separated from the clot activator vial by centrifugation. Rose Bengal plate test (RBPT), the OIE recommended screening test for brucellosis using coloured antigen (Indian Veterinary Research Institute, Bareilly, India) was performed [13]. Serum sample was also screened for antibodies against *Brucella* using commercial i-ELISA kit based on the purified LPS antigen (ID-Vet, France) according to the manufacturer's instructions.

**Bacteriological evaluation**

Isolation and identification was resorted for finding the specific *Brucella* species and to ponder the epidemiological link involved. Blood sample was immediately processed by lysis centrifugation blood culture method [14]. Aborted fetal membranes and fetal stomach contents were individually ground in approximately 10% (w/v) sterile phosphate buffered saline (PBS, pH 7.2) using sterile sand. The vaginal swab, processed blood sample, aborted fetal membranes and fetal stomach contents were inoculated into *Brucella* broth (Himedia, M348) with added *Brucella* selective supplement (Himedia, FD005) and 5% inactivated horse serum. The inoculated broths were incubated for 7–10 days at 37 °C with $CO_2$ for capnophilic species like *B. abortus* and without 5% $CO_2$ for non-capnophilic species like *B. suis* [15]. A loopful of the broth cultures showing turbidity after incubation were inoculated onto Tryptose Agar Base media (Himedia, M996) supplemented with *Brucella* selective supplement. Colonies suspected for *Brucella* were identified on the basis of colony morphology, growth characteristics, routine staining by Ziehl Neelson staining and biochemical methods [16] like $CO_2$ requirement, Urease test, Oxidase test, Catalase test, $H_2S$ production test, Thionin and Basic fuchsin dye reduction test.

**Molecular evaluation**

The QIA amp DNA extraction kit (Qiagen, cat no.69504) was used for isolation of genomic DNA from *Brucella* culture according to the manufacturer's instructions. Multiplex AMOS polymerase chain reaction (AMOS PCR) technique [17] was carried out in a 25µl reaction with 1µl of each primer in a cocktail of five primers, 12.5µl of Taq DNA Pol 2X Master Mix Red (Ampliqon IIII) and 1µl bacterial DNA (100–200 ng/µl). Following a 10-min activation at 95 °C, reaction preparations were cycled in a thermocycler for 30 cycles consisting of 30 sec at 94 °C, 30 sec at 57 °C, and 120 sec at 72 °C and final extension for 5 min at 72 °C. PCR products were analysed by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

**Results and Discussion**

Serum sample of the aborted sow showed positive results in both RBPT and i-ELISA as it revealed presence of agglutination and positive OD values respectively. *Brucella* colonies were identified from all the inoculated samples of the sow as translucent colonies with a pale honey colour when viewed in the daylight and convex pearly white when viewed from above. The strain isolated was typed as *B. abortus* biovar 1 by biochemical assays. DNA of *B. abortus* biovar 1 was amplified from the culture samples by AMOS PCR as depicted in Figure 1.

In this study, swine brucellosis was confirmed with unexpected detection of *B. abortus* as the causative agent, though *B. suis* was the usual etiological agent causing infection in porcine species. It was found that in areas where cattle are maintained, *B. abortus* was of major concern for domestic pigs rather than *B. suis* [18]. Similar to the present study, an enzootic *B. abortus* infection was reported in a feral swine herd suggesting that feral swine may act as a reservoir for *B. abortus* as well as *B. suis* for domestic livestock [9]. The existence of brucellosis in wildlife such as boars, bison, hare etc. may cause spillover transmission of brucellosis to domestic swine herds reared in outdoor breeding systems [19]. In integrated farming, keeping swine together on pastures or in pens with ruminants and other animals allowed them the opportunity to eat aborted fetuses and placenta causing vulnerability to brucellosis [19]. Similarly, pigs may be susceptible to *B. melitensis* infection of small ruminants, which could easily occur in the area where pigs are bred in open-air farms [20]. There is increased potential for disease transmission, which may impact humans, domestic swine and wildlife due to the free range systems, expansion of home range by feral swine (*Sus scrofa*) populations scavenging the organic waste generated due to the anthropurgic nature of mankind (6,22).

The findings in the study showed tangible evidence of cross-species transmission of *B. abortus* in an aborted sow calling out a plausible explanation for spillover transmission of *Brucella* species from domestic or wild ruminants. Being identified as the case of abortion due to *B. abortus* infection, the owner was advised to isolate and cull the animal to avoid the spread of infection to other animals. Strict disinfection of the premises, biosecurity methods and serosurveillance of the other animals in the holding were also advocated. It also suggests that the presence of *B. abortus* in pigs may not only highlights the zoonotic risk to farmers, butcher, abattoir works and pork consumers but also portrays the need for investigation of the epidemiology, transmission dynamics and pathogenicity of the organism in pigs and man.

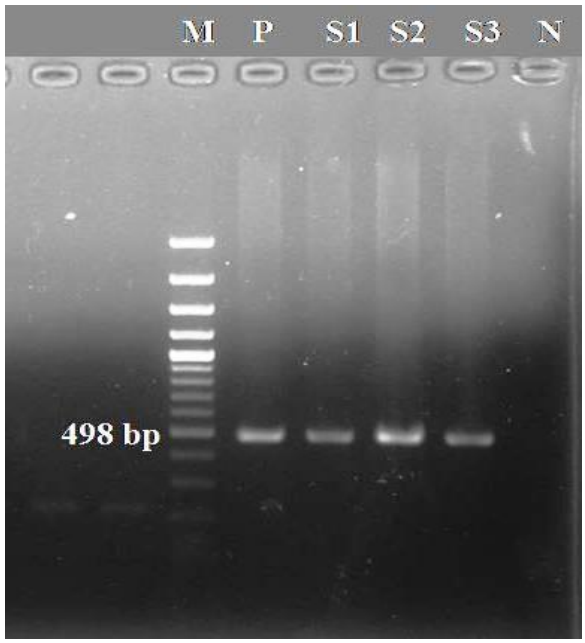


**Fig 1:** AMOS PCR with Lane M as 100 bp ladder, Lane P as positive control, S1-3 are culture samples from vaginal swab, aborted fetal membranes and fetal stomach contents respectively, Lane N as negative control. Lane P, S1-3 showing specific bands at 498 bp confirming presence of *B. abortus*

## Conclusion

It is concluded that cross-species transmission of *B. abortus* to swine is established showcasing the breach in species specificity of *Brucella* species. Strict serosurveillance of brucellosis in domestic and feral pigs is needed to establish an epidemiological map. It can also be stated that appropriate diagnostics for swine brucellosis from point of care to confirmatory needs to be developed and commercialized.

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