

## Seroprevalence of Brucellosis from Pigs: The First Report in Central Ethiopia

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### Abstract

A cross-sectional study was carried out to determine seroprevalence of brucellosis from pigs in Central Ethiopia from October, 2013 to May, 2014. A total of 553 blood samples were collected and sera were tested with Rose Bengal Plate Test (RBPT). Out of 553 sera 25 (4.5%) were positive for *Brucella* antibodies. The results showed that higher seroprevalence in young (<12 months) (5.9%) and male (8.2%) compared to adult (≥12 months) (3.6%) and female pigs (1.6%). The highest positive rates were obtained from pigs originated from Adama area (13.1%) followed by pigs from Addis Ababa area (8.3%). The study showed statistically significant difference ( $P<0.05$ ) in seroprevalence between origin and sexes of pigs considered. Our study demonstrated the first report of brucellosis from pigs in Central Ethiopia. Therefore further detailed investigations are needed as brucellosis has significant economic implications in pigs and public health importance.

**Keywords:** Brucellosis; Pig; RBPT; Central ethiopia

### Introduction

Brucellosis is a serious zoonotic disease affecting man and all domestic animals including pigs. It is considered as one of the great public health problem all over the world [1]. There are five biovars of *B. suis* with biovars 1, 2 and 3 of *Brucella suis* infect primarily domestic and feral pigs, as well as wild boar (*Sus scrofa*) [2-4]. Brucellosis in pigs is a chronic disease most often characterized by infertility and abortion in sows and by orchitis in boars [5]. The disease has drastically affected pig production through abortion, birth of weak/unthrifty piglets, infertility and orchitis in the boar and these constitute serious economic losses [6]. Extragenital lesions such as lymphadenitis, subcutaneous abscesses, arthritis, and spondylitis are also common [7]. In humans, the infection is usually confined to those who are occupationally exposed to pigs, and to laboratory workers. The capability of *B. suis* to colonize the bovine udder with subsequent shedding in milk, has the potential to be a serious human health risk [8].

The transmissions for *B. suis* are similar to those identified for other types of *Brucella* infection, being essentially the oral, nasopharyngeal, conjunctival and vaginal mucosa. There is generally a relatively long incubation period before clinical signs appear. These are not usually visible in young animals, and their occurrence will depend mainly on the age, sex and physiological state of animals at the time they are infected. As an example, animals infected during critical periods of the pregnancy (the first third to half of pregnancy) will abort approximately 30 to 45 days after infection. However, animals infected at full term do not abort. Similarly animals that are infected before the pregnancy period do not abort during their next pregnancies [9]. Infected pigs excrete *Brucellas* in urine, sperm, vaginal discharge, milk, and also by placenta, lochial secretion, aborted fetuses and the content of subcutaneous brucellosis abscesses [10].

Swine brucellosis has been reported in domestic pigs 3% in Greece [11] and 6.7% in Bangladesh [12]. Other reports from Europe has also indicated, presence of brucellosis affecting wild pigs in Germany, Croatia, Spain, Switzerland and Belgium [11,13-15]. Previous studies

have showed that *Brucella* infections are widely distributed in domesticated animals especially in the developing world [16].

The overall laboratory diagnostic regime techniques for *Brucella* are bacteriological, serological, phenotypic grouping and molecular based methods [17]. To date, even though, none of the serological tests has been shown to be reliable in routine diagnosis in individual pigs; indirect and competitive enzyme-linked immunosorbent assays (ELISAs), Rose Bengal test (RBT), complement fixation test (CFT) and fluorescence polarisation assay (FPA) are the prescribed tests for international trade purposes [8].

Report has indicated that no study was done to determine prevalence of brucellosis (*Brucella suis*) in pigs in East Africa and the occurrence and epidemiology of brucellosis in pigs was poorly understood [18]. In Ethiopia, although the population of pigs has shown increment from 19,000 in 1980 [19] to 29,000 [20], in the industry is still at its infancy. In the past years adequate emphasis was not given for the sector. Unlike other livestock distribution, swine farms are predominantly found in the central part of the country particularly, in Addis Ababa and its surroundings [21]. Several studies have attempted to determine the seroprevalence of brucellosis in ruminants and humans in some regions of the Ethiopia with variable of estimates depending mainly on the analyzed host species, geographic localization, and the serological technique used [22-24] and brucellosis is among the major diseases that seriously hampers livestock industry with significant reproductive

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losses in the country. Brucellosis has not been reported in domestic pigs in Ethiopia. Therefore the objective of this study was to describe the status of brucellosis in domestic pigs using Rose Bengal Plate test in Central Ethiopia.

## Materials and Methods

### Description of study area and animals

The study was conducted in pig farms and pigs brought at Addis Ababa slaughterhouse from the three purposively selected sites in Central Ethiopia namely Addis Ababa, East Shewa (Bishoftu and Adama) and West Shewa (Ambo area) (Figure 1). The selections of those study areas were mainly based on availability of pig farms and easy of accessibility in terms of distance from Bishoftu College of Veterinary Medicine and Agriculture. The majority of pigs considered for this study were from Bishoftu because many large and small scale commercial pig farms are concentrated around this area.

Bishoftu and Adama are found in East Shewa Zone of Oromia Regional State at a distance of 45 and 100kms from Addis Ababa respectively. Bishoftu is situated at a longitude of 38°58' E to 39°22'E and latitude of 08° 22'N to 8° 56' N. The altitude of the district ranges from 1500 to over 2000 meters above sea level (masl). The area is characterized by a tepid to cool sub moist agro- ecology. The average rainfall is about 839 mm, while the mean minimum and maximum temperatures recorded for 27 years ranged from 7.9 °C to 28 °C with an overall average of 18.5°C (IPMS, 2004). Adama is situated 1600-1700 masl and in a latitude from 8°-33.8N to 8°-36N and a longitude from 39°11' 57E to 39°21'15E in the Rift valley of warm climate. Its average annual weather conditions are 21°C with small amount of rainfall from

June to September. In general, the town has sunny and windy weather conditions [25].

Ambo district is found in West Shewa Zone of Oromia Regional State. The area is found at a longitude of 37° 32' to 38° 3' E, and latitude of 8° 47' to 9° 20' N and the altitude within the district ranges from 1400 to 3045 masl. The climatic condition of the area is 23% highland, 60% midland and 17% lowland with an annual rainfall and annual temperature ranging from 800-1000 mm and 15-29°C, respectively. The mean temperature is 18.6 °C. The rainfall is bi-modal with the short rainy season from February to May and long rainy season (over 58.8% of the total annual rainfall) from June to September. Agriculture, of mixed type, is the main occupation of the human population in the area. Major livestock reared include cattle, sheep, goats, poultry, and pack animals (mules, horses, and donkeys) [26].

Addis Ababa is the capital city of Ethiopia. It covers about 540 km<sup>2</sup>, of which 18.2 km<sup>2</sup> is rural. The city is located at 9°1' 48 "N and 38° 44' - 24" E at an average altitude 2,200 of 3300 masl. The annual rainfall is about 800-1100 mm and a mean annual maximum and minimum temperature is about 21°C and 27°C respectively [27].

### Study designs and sample size

A cross-sectional study design was carried out in Central Ethiopia from October, 2013 to May, 2014. A total of 553 individual pigs were sampled from purposely selected study areas. For blood collection on farm basis and Addis Ababa abattoir systematic random sampling technique was used. Since there was no previous study of brucellosis in pigs in Ethiopia the sample size was calculated according to Thrusfield [28] by considering 50% expected prevalence (*P*) and 95% confidence interval (*Z*=1.96) with a 5% desired absolute precision (*d*) using the

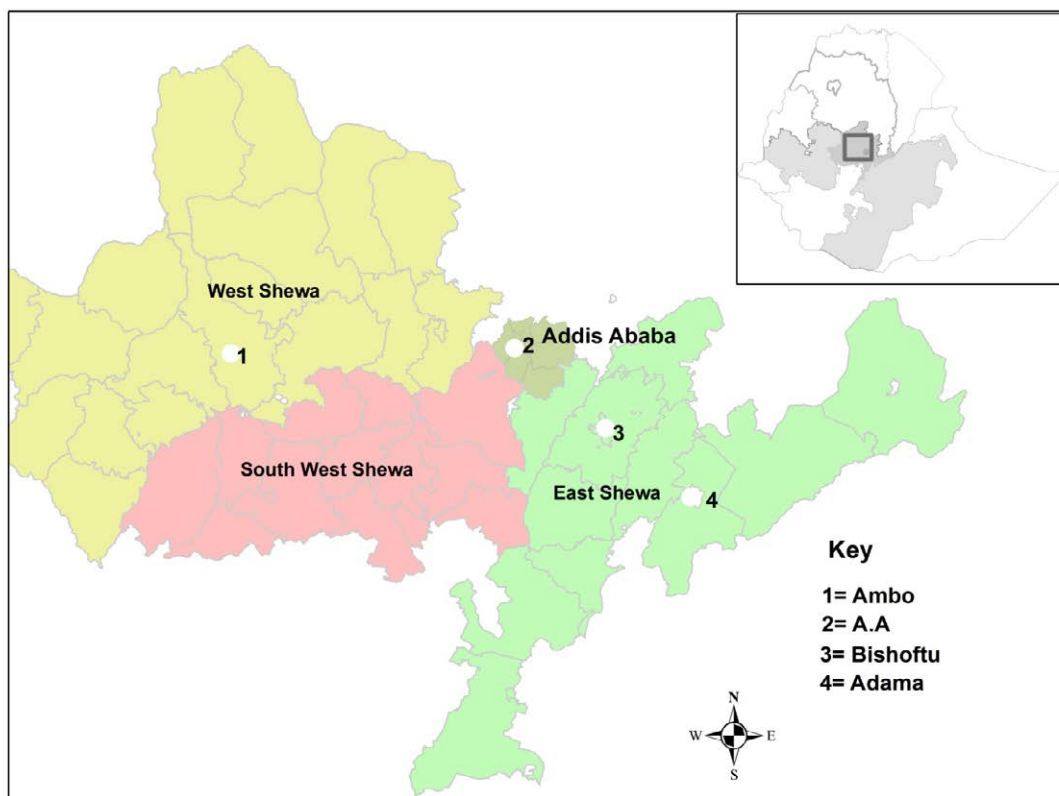


Figure 1: Study areas West Shewa (Ambo), Addis Ababa (A.A) and East Shewa (Bishoftu and Adama).

formula  $N=(Z)^2 P(1-P)/d^2$ . The calculated required sample size (N) was 384. However, total number of sampled animals was increased to 553 for better accuracy.

The abattoir was selected because slaughtered animals are obtained from all areas of the country, including from large commercial and small scale producers and the meat is sold locally by supermarket owners to consumers. The number of samples was determined by the number of animals that were slaughtered on the days of the visits and a total of 290 pigs were sampled where pigs were mainly originated from Adama, around Addis Ababa area and Bishoftu. Information recorded included sex, age and animal origin when available. Out of total pigs sampled, blood samples of 184 pigs from Bishoftu, 55 pigs from Ambo and 24 pigs from Addis Ababa Akaki kaliti sub-city were sampled from live animals in the farm.

### Collection of blood samples

Blood samples were taken by venipuncture from the ear vein on farms after proper restraining of pigs and disinfected with alcohol whereas at abattoir from jugular vein at the time of slaughter with sterile plain vacutainer tubes. The samples were transported to Ethio-Belgium laboratory, College of Veterinary Medicine and Agriculture, with ice pack. Sera were collected following centrifugation at 300 rpm for 10 minutes and transferred to the sterilized labeled eppendorf tube. The sera were kept at -20°C until tested for antibodies against natural *Brucella* infection using Rose Bengal Plate Test (RBPT).

### Serological tests

*Brucella* antigen along with positive and negative control sera were obtained from National Veterinary Institute (NVI), Ethiopia. Rose Bengal Plates Test (RBPT) was performed according to World Organisation for Animal Health (OIE) standard procedures [8]. Sera with strong agglutination were considered as positive for brucellosis. Accordingly, the formation of distinct pink granules (agglutination) was recorded as positive, while the absence of agglutination was recorded as negative.

### Data management and analysis

The collected data and serological test result were entered into Microsoft Excel spreadsheet. The relationship of associated risk factor with positive serological test result was analyzed with Chi-square test using SPSS 20. A test value considered as statistically significant when  $P<0.05$ .

### Results and Discussion

Twenty five (4.5%, 95% CI: 0.028-0.063) out of the 553 pigs tested were positive for *Brucella* antibodies with RBPT. The overall seroprevalence rate found in the present study was lower as compared with the findings of 6.7% from Bangladesh [12]. Similarly it was much lower than the seroprevalence report from Nigeria (30.6%) [29]. In contrary the results of the present study was relatively higher than the seroprevalence reported from Greece (3%) in intensively managed swine herds [11]. The variation in this seroprevalence might have been due the disparity in geographical locations, climatic conditions and management practices in the different study areas. Other studies have also indicated that the rate of brucellosis infection varies among pig herds, from farm to farm or by country [30-32] by origin of tested pigs (wild or domesticated) [30] and by testing method used [33]. Furthermore, contact with other animals was also reported to be major risk factors that were influencing the occurrence brucellosis [34].

With regard to the age of the animal higher seroprevalence was observed in young (< 12months) (5.9%) as compared to adult ( $\geq$  12 months) pigs (3.6%). This result was disagreement with the findings of Rahman et al. [12] who found higher prevalence of brucellosis in aged animal (8.1%) than young (0.0%). Previous study by Leite et al. [34] identified that young age of the animals as risk factor that was influencing the occurrence brucellosis.

The results of the present study showed that higher seroprevalence of brucellosis in female 8.2% (20/244) than male 1.6% (5/309) with significant association ( $P< 0.005$ ). This finding was in agreement with the observation of Rahman et al. [12] who found a high prevalence of brucellosis (7%) in female and 5.9% in male pigs in Bangladesh. Similar observation was also recorded by other investigator in wild boar (*Sus scrofa*) from Republic of Croatia [5]. In contrast to this finding Ngbede et al. [29] found relatively higher prevalence of brucellosis in males than female pigs in Nigeria. The higher rate of infection in females might be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate.

The study showed highest prevalence in pigs originated from Adama area followed by Addis Ababa area and statically significant ( $P<0.05$ ) variation was observed among study areas (Table 1). The high prevalence found in Adama and Addis Ababa area might be associated with climatic condition where Adama is located at hot lowland of Rift Valley as compared Addis Ababa situated at highland with humid climate. Previous studies have showed that *Brucella suis* is moderately influenced by environmental factors, with the microorganism's survival time in the environment decreasing as temperatures rise. However, the bacteria often survive desiccation and can survive freezing temperatures for over two years. Facilities and pasture can remain contaminated for long periods but direct sunlight reduces the bacteria's survival significantly [4]. Pigs originated from Addis Ababa area were mainly kept under intensive and extensive management system. Earlier studies have indicated that the main risks associated with the occurrence of brucellosis in pig farms are; introduction of infected animals [35], contact with other animals although domestic pigs are infected mainly by *B. suis*, less frequently they may become infected with *B. abortus* or *B. melitensis* in regions where brucellosis is endemic in cattle or small ruminants [36]. In general this study provides important information regarding the status of pig brucellosis in Central Ethiopia and the findings indicated that brucellosis is an important disease of pigs and public health concerns. Furthermore, pigs could serve as reservoirs for brucellosis and could be a potential risk of brucellosis transmission for other domestic animals.

Variables	Total number	Number positive (%)	P-value
Age			0.147
Young $\leq$ 12 months	221	13 (5.9)	
Adult > 12 months	332	12 (3.6)	
Sex			<0.001
Females	244	20 (8.2)	
Males	309	5 (1.62)	
Origin			<0.001
Ambo	54	0	
Bishoftu	246	1 (0.4)	
Addis Ababa area	192	16 (8.3)	
Adama area	61	8 (13.1)	
Total	553	25 (4.5)	

Table 1: Seroprevalence of brucellosis in pigs based on age, sex and origin.

## Conclusions

The high seroprevalence of brucellosis observed in central Ethiopia suggested the importance of this zoonotic disease in pig production and public health. Thus, this study warrants extensive investigations to elucidate the animal and human health significance of brucellosis in the study area. Further research is required to investigate its economic importance in pig production, due to losses from abortion and infertility. Furthermore, future research is also needed to identify the types of *Brucella* spp and biotype affecting pigs and the underlying risk factors of brucellosis.

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