



## Sero-Prevalence of *Bordetella bronchiseptica* in Pigs from Some Selected Farms in Abeokuta, Ogun State

Durosinmi, E. M.<sup>1</sup>; Olufemi, F. O.<sup>1\*</sup>; Oyekunle, M. A.<sup>1</sup>; Omotainse, O. S.<sup>2</sup>; Omoshaba, O. E.<sup>1</sup>; and Akinkuotu, O. A.<sup>1</sup>

<sup>1</sup>Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria; <sup>2</sup>Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria; \*Corresponding author: Email: woleolufemi84@gmail.com; Tel No: +2347080488340

### SUMMARY

*Bordetella bronchiseptica*, the causative agent of atrophic rhinitis in pigs, is a Gram-negative coccobacilli that colonizes the ciliated epithelium of the respiratory tract of animal and humans. The dearth of information on *B. bronchiseptica* in Abeokuta, Ogun State, Nigeria and the emerging zoonotic importance of *B. bronchiseptica* as an agent of respiratory infection in humans gave credence to the need to investigate presence of this organism in our livestock. Serum samples (227) were collected from pigs of both sexes and different ages. Indirect ELISA technique was used to detect the presence of *B. bronchiseptica* antibodies in the serum samples. The prevalence of *B. bronchiseptica* in pigs of different age groups and sexes were compared using chi-square test and significant level was set at  $p < 0.05$ . The sero-prevalence rate of *B. bronchiseptica* in pigs was 19.4% (44/227). All (100%) age groups of pigs were sero-positive. The sero-prevalence rate of *B. bronchiseptica* in females was significantly higher ( $p < 0.05$ ) than the rate recorded in males. The sero-prevalence rate was significantly lower ( $p < 0.05$ ) in the piglets (9.3%) than in growers (35.1%), adults (30.5%) compared to weaners (21.7%). This study revealed an increase in sero-prevalence of *B. bronchiseptica* with age in pigs reared in Abeokuta.

**Key words:** Sero-prevalence, *Bordetella bronchiseptica*, Pigs, Ogun State.

### INTRODUCTION

Swine production plays a vital role in food security, poverty alleviation, and employment generation in Nigeria (Adebisi, 2011). Swine production among other species has a high potential to contribute to high economic gain, this is because pigs have high fecundity, high feed conversion ratio, early maturing, short generation interval and relatively small space

requirement (Lekule and Kyvsgaard, 2003) providing 44 % of meat in the world market (FAO, 2001).

The natural habitat of *B. bronchiseptica* is the upper respiratory tract of healthy and diseased humans, pigs, dogs, rabbits, guinea pigs, rats, horses and cats. The most frequently affected species are pigs, dogs and cats. *B. bronchiseptica* is involved in the

development of the atrophic rhinitis in pigs (Quinn *et al.*, 1994). Atrophic rhinitis is an infectious disease of swine characterized by serous to mucopurulent nasal discharge, shortening or twisting of the snout, atrophy of the turbinate (conchal) bones and reduced productivity. Infections with *B. bronchiseptica* alone can cause a mild to moderate form with non-progressive turbinate bone atrophy Organisation of International Epizootics - OIE, (2012). A large proportion of apparently health pig herds may be infected with *B. bronchiseptica* or non-toxigenic *P. multocida* and show a mild degree or low prevalence of turbinate atrophy (OIE, 2012). Bordetellosis caused by *Bordetella bronchiseptica* in pigs is an economically important disease because infected pigs show a 6-10% reduced daily weight gain (Donko *et al.*, 2003). Although usually considered an opportunist or secondary invader, *B. bronchiseptica* can cause pneumonia and atrophic rhinitis with turbinate deformation in growing pigs (Kumar *et al.*, 2014).

*Bordetella bronchiseptica* is acquiring relevance because of its increased importance as a human pathogen (Woolfret and Moody 1991; Bauwens *et al.*, 1992). Most cases in humans are in immunocompromised, especially Human Immunodeficiency virus (HIV)-infected patients where it has recently been documented to cause severe pulmonary infections (Rampelotto *et al.*, 2016).

Antibiotic resistant strain of *Bordetella bronchiseptica* had also been isolated in dogs and cats with respiratory tract infection across Europe (Morrisey *et al.*, 2016).

The emerging zoonotic potential of *B. bronchiseptica* gives credence to the need to study the presence of this organism in our livestock system.

Data from this study will be useful to both the scientific community and policy makers on the status of *B. bronchiseptica* in Abeokuta; Ogun State with respect to the

presence of the pathogen in food animals especially pigs. The objective of this work is to detect *Bordetella bronchiseptica* antibodies in the blood of the studied pigs using ELISA technique and to compare the prevalence in pigs of different age groups and sexes.

## MATERIALS AND METHODS

The samples were collected from apparently healthy pigs in Abeokuta in Ogun State, Nigeria from 2014 to 2015. Abeokuta is located on Latitudes 7° 5' N to 7° 20' N and Longitudes 3° 17' E to 3° 27'E and is located in the sub-humid tropical region of South-western Nigeria. The city enjoys a tropical climate with distinct wet and dry seasons with dry period of about 130 days. The study was cross-sectional. The farms used are located in the two main local government areas namely Abeokuta South and Abeokuta North Local Government Areas. Stratified random sampling was employed to assign pigs into males and females, and categorized age groups before sampling.

Adopting Cannon and Roe (1982), a prevalence of 15% was taken and the sample size was calculated to be 227 pigs. The animals were grouped according to their age group (Table 1) and total of 227 blood samples were collected under strict aseptic conditions.

The samples were transported to the laboratory as soon as possible using cold pack and sera were harvested from the blood samples. Analyses of the samples using Enzyme Linked Immunosorbent assay (ELISA) method were done at the Department of Veterinary Microbiology and Parasitology (Veterinary Microbiology Laboratory), Federal University of Agriculture Abeokuta, Ogun state. ELISA plates coated with *Bordetella bronchiseptica* lipopolysaccharide antigen (Glory Bioscience, China) were used in Indirect ELISA assay. The procedure was carried out according to the manufacturer's instructions.

The wash buffer was reconstituted by adding 20mL of the wash buffer into 580mL of distilled water. Fifty microliters of the positive and negative controls were assigned into different wells. Forty microliter (40µl) of the sample diluent was dispensed into the test wells followed by addition of 10µl of the test sample.

The plate was then sealed with the membrane and incubated at 37°C for 30 minutes. Thereafter, the plate membrane was removed; the contents of the wells were discarded and dried. The wash buffer was added to each well and left for 30 seconds before draining. This was repeated for five times. The incubation step was repeated and the washing step was repeated. Fifty microliters of the mixture of chromogen solution A and chromogen solution B (Thermo Fisher Scientific) were added to each well. It was then incubated in the dark for 15minutes at 37°C. Fifty microliters of the stop solution (0.16M sulfuric acid) was added to each well. The plate was read within 15 minutes of the addition of stop solution using an ELISA reader (BioRad) at 450nm wavelength.

The cut off is taken as the Optical Density (OD) of the negative control plus 0.15 as stipulated by the manufacturer. The wells with OD equal to or greater than the cutoff were taken as the positive.

### Statistical Analysis

Data collected were analyzed using on the Statistical Package for Social Sciences (SPSS) version 16 on windows 7 Operating System. Chi-square test was used to compare the detection rates in different sexes and age categories of pigs.

### RESULTS AND DISCUSSION

Of the 227 sera analyzed, 44 (19.4%) were positive for *B. bronchiseptica*. Both sexes were sero-positive for *B. bronchiseptica* with the females having the higher prevalence rate, 24.4%, than the rate recorded in the males, 13.9% (Table I).

All age groups of pigs were sero-positive for *B. bronchiseptica*. The highest rate, 35.1% (13/37), was observed in growers while the lowest rate, 9.3% (10/108), was observed in the piglets. The sero-prevalence rate recorded in the piglets were significantly lower ( $p < 0.05$ ) than the rates recorded in growers, weaners and adults (Table 1).

In this study, the sero-prevalence of *Bordetella bronchioseptica* in apparently healthy pigs in Abeokuta, Ogun State is reported. Although the disease, atrophic rhinitis, caused by *B. bronchiseptica* in pigs, has not been documented in the study areas of Abeokuta prior this study, the result of this serological detection of the organism corroborated the reports of Parton (2005) and OIE (2012) that the organism is cosmopolitan in distribution.

**TABLE 1:** The sero-prevalence of *B. bronchiseptica* in relation to sex and age of pigs in Abeokuta

Variables	Total No. Samples	Total No. Seropositive (%)	P value
<b>Sex</b>			
Male	108	15 (13.9)	0.046
Female	119	29 (24.4)	
<b>Age</b>			
Piglets	108	10 (9.3)	0.0011
Growers	37	13 (35.1)	
Weaners	46	10 (21.7)	
Adults	36	11 (30.6)	

The sero-prevalence (19.4%) recorded in this study was lower than 42.0% recorded in Clu- Napoca, Romania by Denes and Rapuntean (2007) and 86.3% recorded in Northern India by Sandeep *et al.* (2014) respectively. This was not only because atrophic rhinitis is highly prevalent in those regions but also because the pigs, sampled by these authors, were from extensive system of management.

The age-related sero-prevalence recorded in this study in piglets (9.8%) is lesser than in weaners (21.7%), growers (35.1%) and adult (30.6%). The results agree with the report of Sandeep *et al.* (2014) which suggested that the low prevalence in piglets is due to maternal passive immunity derived from the colostrum of the adult pigs. The higher infection in adult pigs also corroborates the observations of Sandeep *et al.* (2014). This supports the fact that *B. bronchiseptica* is endemic in this region and that the possibility of being infected with the organism by the carrier older pigs increases with time. The results obtained from apparently healthy pigs from this study shows that there is a potentially high risk of outbreak of atrophic rhinitis in pigs in Abeokuta with the attendant economic losses that are associated with the disease in pigs. Although sero-prevalence of the organism in humans that are constantly in contact with these animals is not covered by this study, there is high potential of transmission of the zoonotic pathogen to humans.

The observation that females had higher infection rate than males in this study was at variance with the report of Sandeep *et al.* (2014) but supports the report of Songer and Post (2005) and Nicholson *et al* (2016). This could be because the females are retained

longer on the farm for breeding and the practice employed by pig farmers in which one boar is introduced to service several sows may have predisposed the latter to the infection.

The serological detection of the organism in asymptomatic pigs supports the reports of Songer and Post (2005) who reported that although *B. bronchiseptica* is frequently isolated from young pigs in outbreak of atrophic rhinitis, the infection also occurs widely in herds without this condition. The authors also suggested that single infection with *B. bronchiseptica* can cause a mild to moderate form of the disease, with non-progressive turbinate bone atrophy and that a large proportion of apparently normal pig herds infected with *B. bronchiseptica* show a mild degree or low prevalence of turbinate atrophy.

## CONCLUSION

The result of this study suggests that subclinical infection with *B. bronchiseptica* is highly prevalent in pigs reared in Abeokuta, Ogun state. It is therefore important to consider the organism as a treat to other domestic and wild animals and humans living in the study area.

The ELISA, although is expensive, is faster and may be more suitable for large-scale epidemiological surveys. While the study was localized in Abeokuta, Ogun State, it is suggested further large scale epidemiological studies are carried out across Nigeria to determine the prevalence of the pathogen in the country. Further molecular characterization of the isolates is also required to ascertain the strains of *B. bronchiseptica* that are in circulation in Nigeria.

## REFERENCES

- ADEBAMBO, O. (1995): Selection and handling of pig breeds for profitable pig farming in Nigeria. In: Pig production Workshop Training Manual pp215. NAERLS, ABU, Zaria.
- ADEBISI, O.R. (2011). Gastrointestinal helminths and public health: Overview of a neglected sector.

- International Journal of Veterinary Medicine*, Vol. 4(2).
- BAUWENS, J. E., SPACH, D. H., SCHACKER, T. W., MUSTAFA, M. M., and BOWDEN, R. A. (1992): *Bordetella bronchiseptica* pneumonia and bacteremia following bone marrow transplantation. *Journal of Clinical Microbiology*, Vol. 30(9): 2474-5.
- CANNONNON, R. M. and ROE, R. T. (1982): *Livestock Disease Surveys: A Field Manual for Veterinarians*. Australian Government Publishing Service, Canberra
- DENE, A. L., and RAPPUNTEAN, G. H. (2007): Detection of *Bordetella bronchiseptica* infection in swine using agglutination test. *Bulletin USAMV Cluj-Napoca, Seria, Med. Vet.*, vol 67, Edit. Academic Press 1-2.
- DONKO, T., KOVACS, M., and MAGYAR, T. (2003): The effect of atrophic rhinitis (AR) on the weight gain of swine, "Agriculture conspectus scientificus, vol. 68, pp. 161 – 164.
- FOOD AND AGRICULTURE ORGANISATION (FAO). (2001): *Food. Bulletin of Statistics*. Vol.2 (1): 1-5
- KUMAR, S., SINGH, B. R., BHARHARWAJ, M., SINGH, V (2014). Occurrence of *Bordetella* infection in pigs in northern India. *Int. J Microbiol*. Published online Jan. 12 doi: 10.1155/2014/238575
- LEKULE, P. F. and KYVSGAARD, N. C. (2003): Improving pig husbandry in tropical resource-poor communities and its potential to reduce risk of porcine cysticercosis. *Acta Tropical*, 87: 111-117.
- MORRISSEY, I., MOYAERT, H., de JONG, A., EL-GARCH, F., KLEIN, U., AND LUDWIG C (2016). Antimicrobial susceptibility monitoring of bacterial pathogens isolated from respiratory tract infections in dogs and cats across Europe. *Vet. Microbiology*. Vol. 191 (15): 44-51
- NICHOLSON, T.L., SHORE, S.M., REGISTER, K.B., BAYLES, D.O., KINGSLEY, R.A., BRUNELLE, B.W (2016). Comparative genomic analysis of the swine pathogen *Bordetella bronchiseptica* strain KM22. *Vet Microbiol*.182: 87-94
- OIE, (2012): Organization for International Epizootics, *Terrestrial Manual*, Atrophic rhinitis of swine. Version adopted by the World Assembly of Delegates of the OIE in May 2012, Chapter 2.8.2.
- QUINN, P.J., CARTER, M.E., MARKEY, B., CARTER, G.R. (1994): *Clinical Veterinary Microbiology*, Wolf Publishing, 280-283.
- RAMPELOTTO, RF., HORNER, A., HORNER, C., RIGHI, R and HORNER, R.(2016): Pneumonia caused by *Bordetella bronchiseptica* in two HIV-positive patients. *Sao Paulo Med J*. 134(3):268-72.
- SANDEEP, K., BHOJ, R. S., MONIKA, B and VIDYA, S. (2014): *International Journals of Microbiology*. Article ID 238575.
- SONGER, J. G. and POST, K. W. (2005): *Bacterial and Fungal Agents of Animal Disease: Veterinary Microbiology*. Elsevier Inc. St Louis Missouri, 147-153.
- WOOLFREY, B. F., and Moody, J. A. (1991): Human infections associated with *Bordetella bronchiseptica*. *Clin. Microbiol Rev.*, 4(3): 243-55.