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Seroprevalence of Contagious Bovine Pleuropneumonia in Cattle Slaughtered from Sokoto and Zamfara States, North-western Nigeria

[1*]Ahmad, K. H., [1]Mamman, P. H., [1]Adamu, J., [2]Bello, M., [3]Olorunshola, I. D., [1]Umar, B. N., [1]Dalis J. S., [1]Salawudeen, M. T. and [4]Sada, A.

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria
[2]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria
[3]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Kwara State
[4]National Veterinary Research Institute, Vom, Plateau State, Nigeria

[*] *Author for Correspondence: khahmad@abu.edu.ng*

ABSTRACT

Contagious bovine pleuropneumonia (CBPP), which is caused by *Mycoplasma mycoides* subspecies *Mycoides,* remains one of the most significant challenges to cattle farming in Sub-Saharan Africa, leading to huge economic losses and the exclusion of the affected country from the international cattle trade. This study assessed the seroprevalence of CBPP in cattle presented for slaughter at the major abattoirs in Sokoto and Zamfara States, Nigeria. Exactly 5 mL of blood sample was aseptically collected from each of the 400 heads of cattle via the jugular vein, and sera were then separated and stored at -20°C until the time of analysis. Serological analysis was performed using an IDEXX c-ELISA test kit for CBPP. The optical density of the reactions was read at 450 nm using a Uniequip® plate reader. Seropositive samples had a percentage inhibition (PI) of at least 50%. A chi-square test was applied using SPSS Version 16 for Windows® to determine statistical differences in the disease prevalence rates based on sampling units, cattle sex and breeds. The overall seroprevalence for CBPP was found to be 30.0% in the studied area. In Zamfara State, bulls had a significantly higher (P = 0.001) prevalence rate (32.7%) than cows (32.4%). The results of this study highlight the endemicity of CBPP in Sokoto and Zamfara States; thus, mandatory annual mass vaccinations of cattle against CBPP as well as restricted cattle movement were recommended.

Keywords: Abattoir; Cattle; Contagious bovine pleuropneumonia; Seroprevalence; cELISA

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subspecies *mycoides* (*Mmm*) is an infectious and contagious transboundary respiratory disease of cattle associated with enormous economic losses (Nicholas *et al.* 2008, Wade *et al.* 2015, Sacchini *et al.* 2020, Sada *et al.* 2021). Due to its potential for rapid spread and as one of the major challenges to cattle farming in Sub-Saharan Africa, the disease continues to be a top concern for cattle farmers, veterinarians, and government authorities globally (Nicholas *et al.,* 2008; FAO, 2016; Ahmed *et al.,* 2024; Teshome *et al.,* 2024). Consequently, CBPP-endemic countries are excluded from international animal trades (OIE, 2021). Understanding of the global distribution of CBPP prevalence is essential for developing effective strategies for managing and controlling the condition. Regional cooperation and information exchange between countries and international organizations are crucial for tackling CBPP globally. This could be achieved through knowledge, resources, and best practice sharing, stakeholders can collaborate to lessen the impact of CBPP and protect the global cattle population's health and productivity. The incubation period of CBPP in cattle varies and can be up to six months. During the acute stage of the disease, when the causative agent can rapidly spread, the clinical manifestations include anorexia, fever, and respiratory signs such as dyspnea, polypnea, coughing, and nasal discharges (OIE, 2013; Di Teodoro *et al.,* 2020). While in chronic stage of CBPP, silent persistence of the agent is commonly observed making clinical diagnosis difficult. Distinctive pathognomonic lesions include unilateral pneumonia associated with pleurisy and sequestra (FAO, 2002; OIE, 2021). In the field, CBPP might be confused with other bovine respiratory diseases such as pneumonia due to *Mycoplasma bovis*, pasteurellosis, actinobacillosis, and bovine tuberculosis, among others (FAO, 2002, OIE, 2021). Moreover, in the absence of confirmatory diagnosis during disease outbreaks, antibiotic treatment is normally employed by farmers leading to the development of carrier state (OIE, 2021). These carrier animals are mostly responsible for the persistence of

CBPP in cattle herds and play significant roles in the maintenance and epidemiology of the disease.

Contagious bovine pleuropneumonia has been endemic for several years in Nigeria (Danbirni *et al.,* 2010; Tambuwal *et al.,* 2011) and is causing causes losses that are difficult to quantify. The control of this disease in Nigeria and, by extension, Sub-Saharan Africa has been partially successful owing to the low efficacy of available T1/44 and T1sr vaccines (Yansambou *et al.* 2018; Jores *et al.* 2020), as well as non-implementation of the OIE-recommendations of culling infected animals, and restriction of livestock movement (Yaya *et al.* 2008).

The two serological tests recommended by the world organization for animal health to achieve herd-level serological diagnosis of CBPP, and for rapid disease surveillance in most parts of Europe and Africa are the competitive enzyme-linked immunosorbent assay (c-ELISA) and complement fixation test (CFT) (OIE, 2002). The sensitivity of CFT to *Mmm* infection varies with the clinical stage of CBPP presentation and is greatest in the acute phase due to the elevated level of complement-fixing immunoglobulin in circulation (Amanfu *et al.,* 2000; Thiaucourt *et al.,* 2004; Alhaji and Babalobi, 2016; FAO, 2016). However, in Africa, many herds have adopted c-ELISA-specific monoclonal antibodies that target *Mmm* antigens (Ankeli *et al.,* 2017; OIE, 2018; Sada *et al.,* 2021) due to their almost 100% sensitivity and specificity, and no cross-reactions with other *Mycoplasma* species were reported (Le Goff and Thiaucourt, 1998; OIE, 2018). Therefore, this study was conducted to assess the presence of *Mmm* in cattle slaughtered at the major abattoirs of Sokoto and Zamfara States, Northwestern-Nigeria using cELISA technique.

MATERIALS AND METHODS

Study area

This research was carried out in the two foremost livestock-producing states of Northwestern Nigeria, Sokoto and Zamfara States (MOCIT, 2002; Mamman, 2005). The region is famous for the Sokoto Gudali, Red Bororo, and White Fulani cattle breeds. Sokoto State is situated between longitude 4° 8' E and 6° 54' E, and latitude 12° 8' N and 13° 58' N in northwestern Nigeria. The State is bordered on the north by Niger Republic, on the west by Kebbi State, and on the east by Zamfara State (Figure 1). The State has a total land area of about 32,000 km^2 and a human population of 3,702,676 (NPC, 2006). Sokoto State has the second largest livestock population in Nigeria, with an estimated three million heads of cattle (MOCIT, 2002; Mamman, 2005). Zamfara State is located between latitude 11° 10' N and longitude 6° 15' E, with a total land area of 39,762 km^2 and a human population of 3,582,912 (NPC, 2006). It shares an international border with the Niger Republic to the north, and bordered the states of Kebbi, Kaduna, Sokoto, Niger, and Katsina (Figure 1). The State's cattle population is projected to be around three million, from a total livestock population of over 13 million (MOCIT, 2002; Mamman, 2005). Sokoto Central Abattoir is located within Sokoto North Local Government Area of Sokoto State, Nigeria. While Gusau Modern Abattoir of Zamfara State is located on the outskirts of the state capital (Akawu *et al.* 2018) (Figure 2).

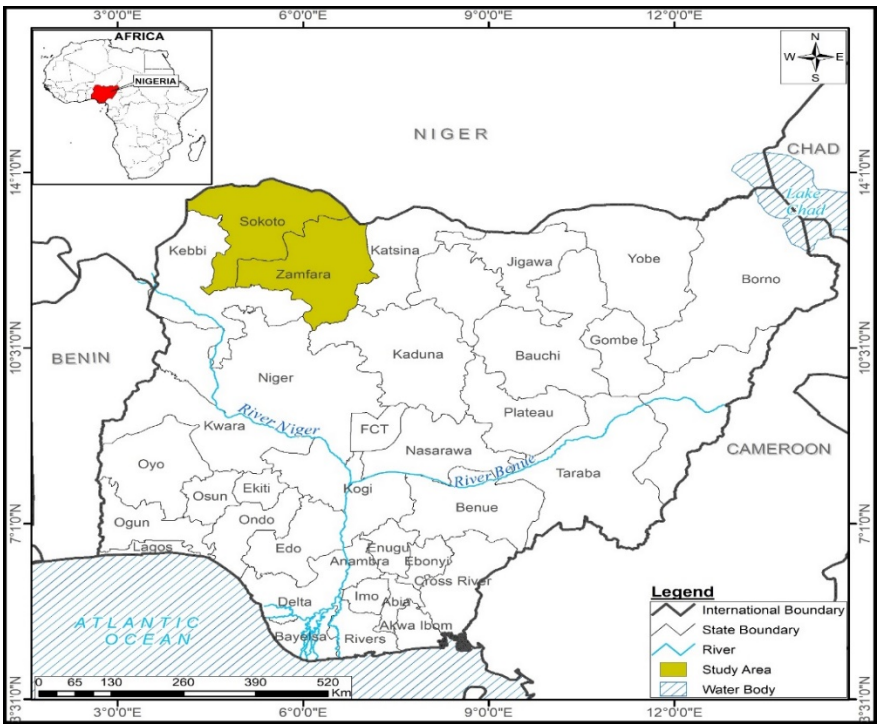

Figure 1: Map of Nigeria showing Sokoto and Zamfara States. Source modified from https://www.openstreetmap.org/#map=6/9.117/8.674

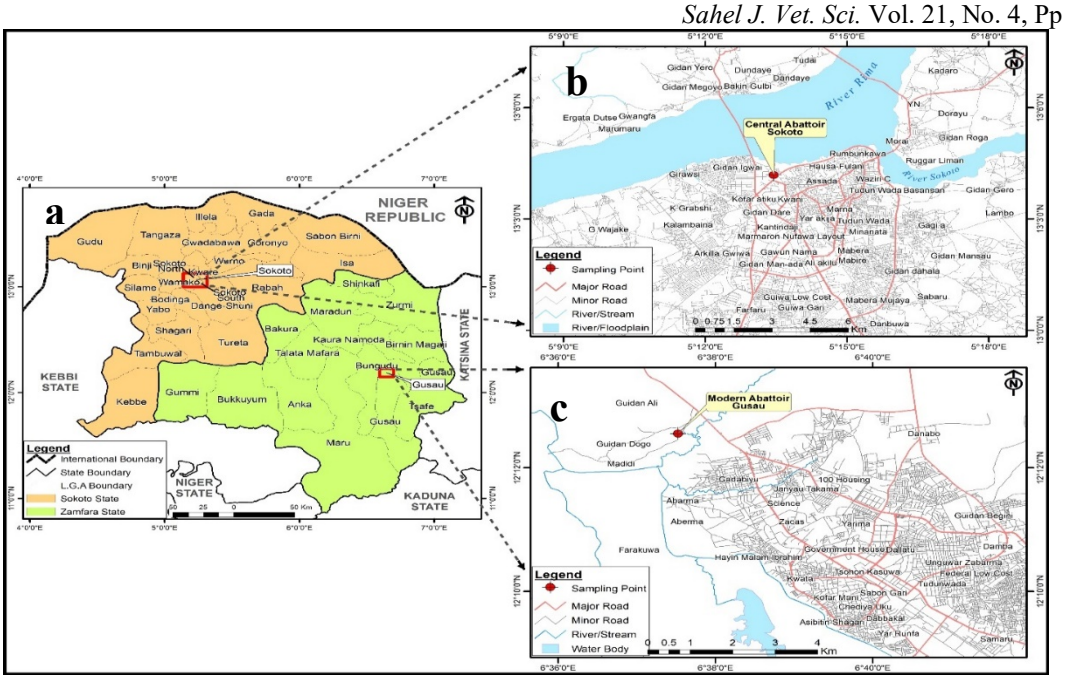

Figure 2: Map of Sokoto and Zamfara States (**a**) showing the Sokoto Central Abattoir (**b**) and Gusau Modern Abattoir (**c**). Source: Modified from https://www.openstreetmap.org/#map=6/9.117/8.674

Study design and Sample size determination

The study design was a cross-sectional study, and blood samples were randomly collected three times per week for a maximum of four weeks per abattoir using a simple random sampling technique. All the blood samples were collected from the two abattoirs during the same period of the year. The required sample size was determined according to the Thrusfield (2005) formula: $n = Z^2 \times P (1 - P) / d^2$, with an expected prevalence of 50% and a desired absolute precision (d) of 0.05 at a 95% (1.96) confidence level. Accordingly, at $1.96^2 \times 0.5 (0.5) / 0.05^2$ the calculated sample size was 384 samples. However, to increase precision, a total of 400 blood sample were collected.

Sample collection

Blood samples were collected in accordance with standard microbiological technique as described by Nicholas *et al.* (2008) and Markey *et al.* (2013). In both abattoirs, 5 mL of blood was aseptically collected per each cattle via the jugular vein at the site of slaughter. Factors such as breed, and sex were also documented. To separate the sera from other components of the blood, the collected blood samples were placed in a slanted position at 4˚C overnight. The sera were collected into labelled sample bottles and packaged in an icepack container for transportation to the Mycoplasma Laboratory of the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, for analysis.

Serological assay

Competitive enzyme-linked immunosorbent assay using IDEXX c-ELISA kit (P05410/10) for CBPP (CIRAD, France) was conducted as per manufacturer's instructions. Briefly, each serum sample was diluted with a specific monoclonal antibody (anti *Mmm* antibody) named Mab 117/5 in a pre-plate. The mixture was then transferred to *Mmm* lysate-coated plate and incubated at 37°C for one hour. The unbound materials were washed with phosphate-buffered saline (300 µL), and 100 µL of anti-mouse conjugate concentrate was then added to each well. This was again incubated at 37℃ for 30 minutes. After incubation, the unbound materials were again washed three times with 300 µL phosphate-buffered saline. Subsequently, 100 µL of TMB enzyme substrate was then added to each well and incubated for another 20 minutes at 37°C. Finally, the reaction was stopped by flooding the plate with 100 µL of a stop solution (sulfuric acid) in each well. The optical densities (OD) of the reactions were then read at 450 nm using a Uniequip® plate reader. Samples were considered positive if the percentage inhibition (PI) was ≥ 50% and negative if the PI was < 50%.

The PI for each serum sample was calculated as follows:

PI = [(MabC OD – Serum OD) / (MabC OD – Cc OD)] X 100

Where OD = optical density, MabC = monoclonal control, Serum = test serum, and Cc = conjugate control. The validity criteria were an OD between 0.5 and 2.0 for Cm; an OD below 0.3 for Cc.

Data analysis

The data obtained from this study were presented in tables in the form of simple descriptive statistics. A chi-square test using SPSS Version 16 for Windows was used to determine any statistical differences in the prevalence rates of the disease based on the sampling units, sexes, and breeds of cattle sampled. Values of $P \leq 0.05$ were considered significant.

Ethical Approval

This study was approved by the Committee on Animal Use and Care of Ahmadu Bello University, Zaria (ABUCAUC) (Approval No. ABUCAUC/2021/079). Administrative permission to collect samples in the two Northwestern States of Sokoto (MAH&FD/VET/161/VOL1) and Zamfara (DAHL/DVPH/021/II) was also obtained before samples were collected. In addition, the study was conducted in

accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

RESULTS

Out of the 400 cattle sera examined for the presence of specific antibodies to *Mmm* using c-ELISA, a total of 120 sera were found to be seropositive giving an overall seroprevalence of CBPP in the study area to be 30.0%. Zamfara State had higher seropositive CBPP cases, 65/200 (32.5%) than Sokoto State, 55/200 (27.5%) (Table 1). However, the difference was not statistically significant (P = 1.190). The Sokoto Gudali breed of cattle was majorly affected, 46/152 (30.3%), followed by White Fulani, 62/205 (30.2%), then Red Bororo, 12/43 (27.9%). Nevertheless, the difference was not statistically significant (Table 2). Similarly, cows had relatively higher CBPP cases (32.7%) than bulls (26.6%), but the variation was not statistically significant (P = 1.255) (Table 3).

The breed-specific seroprevalence of CBPP in Sokoto State was 19.5%, 31.3%, and 50.0% for White Fulani, Sokoto Gudali, and Red Bororo, respectively. Likewise, based on sex, cows (32.0%) had a higher seropositivity for CBPP than bulls (24.8%) (Table 4). However, the difference, were not statistically significant (P > 0.05). In Zamfara State, based on breed distributions, although not statistically significant (P = 5.212), White Fulani cattle had the highest seroprevalence (38.1%) compared to Sokoto Gudali (28.3%) and Red Bororo (17.2%). Interestingly, a significantly higher (P = 0.001) seroprevalence was observed in bulls (32.7%) when compared to cows (32.4%) (Table 5).

Table 1: Seroprevalence of Contagious Bovine Pleuropneumonia in Cattle Slaughtered at Sokoto Central and Gusau Modern Abattoirs, Northwestern Nigeria

Variables	Sokoto	Zamfara	Total	p-value	χ^2
No. collected	200	200	400	1.190	0.275
No. Positive	55	65	120		
Prevalence	27.5%	32.5%	30%		

Table 2: Breeds Specific Seroprevalence of CBPP in Cattle Slaughtered at Sokoto Central and Gusau Modern Abattoirs, Northwestern Nigeria

Breeds	No. Collected	No. Positive	Prevalence	p-value	χ^2
White Fulani	205	62	30.2%	0.101	0.951
Sokoto Gudali	152	46	30.3%		
Red Bororo	43	12	27.9%		
Total	400	120	30%		

Table 3: Sex Specific Seroprevalence of CBPP in Cattle Slaughtered at Sokoto Central and Gusau Modern Abattoirs, Northwestern Nigeria

Sex	No. Collected	No. Positive	Prevalence	p-value	χ^2
Bull	177	47	26.6%	1.255	0.263
Cow	223	73	32.7%		
Total	400	120	30.0%		

Table 4: Breed and Sex Seroprevalence of Contagious Bovine Pleuropneumonia from Cattle Slaughtered at Sokoto Central Abattoir, Sokoto State, Nigeria

Variable		No. Sampled	No. Positive	Prevalence (%)	P-value	X^2
Breed	White Fulani	87	17	19.5	7.538	0.057
	Sokoto Gudali	99	31	31.3		
	Red Bororo	14	7	50.0		
Sex	Bull	125	31	24.8	1.219	0.270
	Cow	75	24	32.0		
Total		200	55	**27.5**		

Table 5: Breed and Sex Seroprevalence of Contagious Bovine Pleuropneumonia from Cattle Slaughtered at Gusau Modern Abattoir, Zamfara Sate, Nigeria

Variable		No. Sampled	No. Positive	Prevalence (%)	P-value	X^2
Breed	White Fulani	118	45	38.1	5.212	0.074
	Sokoto Gudali	53	15	28.3		
	Red Bororo	29	5	17.2		
Sex	Bull	52	17	32.7	0.001	0.973
	Cow	148	48	32.4		
Total		200	65	**32.5**		

DISCUSSION

A comparison of the differences between our findings from Nigeria and the research conducted in Somalia, Ethiopia and Uganda sheds light on the serological prevalence of CBPP in various geographical areas. In this study, the overall seroprevalence of CBPP was observed to be 30.0%, with cows exhibiting a prevalence of 32.7% and bulls at 26.6%. This suggests that although both sexes are impacted by CBPP, cows are marginally more so than bulls. However, Somalian researchers, Ahmed *et al.* (2024) reported a higher total seroprevalence rate of 35.0% for CBPP, with cows having a much higher prevalence of 31.1% compared to bulls' 4.4%. This implies that, just like our work in Nigeria, the impact on cows in Somalia may be more severe. The difference in prevalence rates between this study and the study in Somalia could be attributed to various factors, such as differences in management practices, vaccination coverage, and environmental conditions.

In contrast to the study in Nigeria and study in Somalia (Ahmed *et al.,* 2024), the overall serological prevalence of CBPP is considerably lower in other studies from Ethiopia by Teshome *et al.* (2024) at 9.2%, with cows having 8.7% and bulls having 10.6%, and Uganda by Tweyongyere *et al.* (2024) with an overall CBPP seroprevalence of 25.4%. It's interesting to note that bulls have a higher prevalence rate (10.6%) in Ethiopia than cows (8.7%). This contrasts with our findings and research conducted in Somalia by Teshome *et al.* (2024), where CBPP seroprevalence is often higher in cows. These regional differences in CBPP seroprevalence rates demonstrate the intricate dynamics of CBPP epidemiology, which are impacted by a wide range of variables including geographical location, climate, livestock management practices, and the efficacy of vaccination and other control measures. Knowing these variations can help develop control plans and targeted actions that are suited to the unique requirements of each region.

In Nigeria, CBPP has been the most important endemic economic cattle disease following the official eradication of rinderpest (Olorunshola *et al.,* 2020; Sada *et al.,* 2021). The disease greatly impairs livestock production not only in Nigeria but in sub-Saharan Africa (March *et al.,* 2003; Tambi *et al.,* 2006). Despite the enormous livestock resources, very limited studies on CBPP have been documented in the studied area. The few available data established the presence of CBPP in Sokoto State (Tambuwal *et al.,* 2011; Tambuwal and Egwu, 2017). However, there was no trace of such published information in Zamfara State. Unpublished records from the Zamfara State Directorate of Animal Health and Livestock Development revealed that there wasn't any official mass CBPP vaccination from the state government to curtail this dreaded disease for over a decade.

The 27.5% and 32.5% serological prevalence of CBPP recorded in the present study for Sokoto and Zamfara states was higher than what was reported across the country by many researchers, including 0.63% by Musa *et al.* (2015) in Borno State, 14.39% by Ankeli *et al.* (2017) in Plateau State, 8.75% by Francis *et al.* (2017) in Jigawa State, and 7.3% by Sada *et al.* (2021) in Kastina State. Conversely, the established CBPP seroprevalence rates for the two states were lower than the 47.0%, 56.2%, and 59.4% reported by Danbirni *et al.* (2010) in Kaduna State, Olorunshola *et al.* (2020) in Kwara State, and Anyika *et al.* (2021) in the South-Eastern States. The differences could be attributed to several factors, such as variation in the study period, samples and sampling techniques employed by each researcher, and most importantly, the level of prevention and control strategies instituted by the different states, like annual mass CBPP vaccination and coverage, which have been at least established for Katsina and Borno States by Sada *et al.* (2021) and Musa *et al.* (2015), respectively. The diverse seroprevalence rates highlight the alarming dynamics of CBPP as an important transboundary disease with a proclivity to spread across Nigerian states, especially from the northern region with an abundant livestock population to the other regions. Comparable to the findings of Olorunshola *et al.* (2020) and Sada *et al.* (2021), who both reported in parallel studies that Sokoto Gudali was the most prevalent breed of cattle with 19.4% and 8.51%, respectively, this study established that the Sokoto Gudali breed of cattle was the most prevalent breed affected, with CBPP having the highest seropositivity of 30.3%.

The statistically significant higher CBPP seropositivity observed among bulls (as compared to cows) in the Gusau Modern Abattoir of Zamfara State disagrees with the findings of Olorunshola *et al.* (2020), Anyika *et al.* (2021), and Sada *et al.* (2021). Their findings could be because more cows are retained in the herd for production, and by extension, more are exposed to the *Mmm* antigen. Similarly, in the Sokoto Central Abattoir of Sokoto State, cows had relatively higher (P = 1.219) CBPP seroprevalence than bulls. However, this oppose what was documented by Ankeli *et al.* (2017), This could be because Plateau State rely heavily on the supply of bulls for consumption purposes, so more males are presented for slaughter, increasing the likelihood of having more seropositive among the bulls.

In conclusion, 30% CBPP seroprevalence was found in Sokoto and Zamfara States, Northwestern Nigeria. Zamfara State recorded a higher prevalence of CBPP cases in cattle, with 32.5% testing positive, compared to Sokoto State, which reported 27.5%. Among cattle breeds, the Sokoto Gudali was predominantly affected, with 30.3% testing positive, followed closely by the White Fulani breed (30.2%) and the Red Bororo breed (27.9%). The results of this study highlight the endemicity of CBPP in the Sokoto and Zamfara States; thus, mandatory annual mass vaccination of cattle against CBPP, as well as active surveillance and restricted cattle movement, were recommended.

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors Contribution

AKH collected the sera samples, run laboratory and data analyses, and prepare the first draft of the manuscript. MPH, AJ and BM supervised the work and provided expert guidance. OID conducted some of the sera analyses, while UBN, DJS, SMT and SA assisted with laboratory work and data analyses. All authors contributed to proofreading and preparing the final draft of the manuscript

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