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Paper

Spatial seroprevalence of Foot-and-Mouth Disease in Small Ruminants in Benue State, Nigeria

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Abstract

Foot-and-mouth disease (FMD) presents a significant challenge to the livestock industry and food animal security. In small ruminants such as sheep and goats, FMD infections often remain asymptomatic, which can result in undetected viral transmission across various species. Despite the global significance of FMD, the epidemiological role of small ruminants in its spread remains poorly understood, particularly in specific regions. In Benue State, Nigeria, there is a notable gap in research concerning the seroprevalence of FMD in small ruminants and its associated spatial distribution. 3ABC-trapping enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to non-structural protein (NSP) 3ABC of FMD virus (FMDV) in serum samples of sheep and goats from three local government areas representative of high risk zones of Benue State. The seroprevalence of FMDV in small ruminants was found to be 15.92%, with 14.75% in goats and 19.59% in sheep. Regional differences were also evident, with Zone A (Katsina-Ala) showing seroprevalence of 14.71%, Zone B (Makurdi) at 14.72%, and Zone C (Otukpo) at 19.42%. Overall, our study suggests that small ruminants in Benue State are significantly exposed to FMDV, with prevalence rates comparable to some regions in Nigeria and lower than others, especially when compared to large ruminants like cattle. The findings underscore the necessity for region-specific control strategies, taking into account the dynamics of animal movement and trade routes. Establishing robust surveillance and biosecurity protocols, especially along high-risk trade routes, is essential for reducing the impact of FMDV and ensuring the productivity of livestock farming in the region.

Keywords

Small ruminants, FMDV, NSP, 3ABC-ELISA, Seroprevalence, High-risk zones

Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease that primarily affects cloven-hoofed livestock and wildlife (Ularamu et al., 2020). The disease is endemic in most parts of sub-Saharan Africa (Wungak et al., 2015). The etiological agent of the disease is known as Foot-and-mouth disease virus (FMDV), a positive sense single stranded RNA virus belonging to the genus *Apthovirus* of the family *Picornaviridae,* and it is characterized by high genetic and antigenic heterogeneity (Begovoeva et al., 2023). The genome is surrounded by four structural proteins (VP1-4), and these form an icosahedral capsid (Oem et al., 2004) of approximately 25-30nm in diameter (Grubman & Baxt, 2004). Seven serotypes of FMDV have been identified by cross-protection and serological tests; they are designated O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1. At one time or another, these viruses occurred in most parts of the world, often causing extensive epidemics in domestic cattle and swine (Gickerson, 2017).

Foot-and-mouth disease is classified on the list A of infectious animal diseases by the WOAH, and it is recognized as a major constraint to international trade in livestock and livestock-related products (Oem et al., 2004). The disease is characterized by several clinical signs, most notably fever and the formation of vesicular lesions. These lesions

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commonly appear on the tongue, mouth, muzzle, teats, and feet of affected animals. Over time, the vesicles may erupt, forming painful blisters, which can lead to secondary infections, difficulty in eating, lameness, and significant discomfort for the animals. These clinical signs contribute to reduced productivity and increased morbidity, making FMD a severe concern in livestock management (Gudata, 2015). Though FMD is rarely fatal in adult animals, the morbidity rate during an outbreak is extremely high across affected populations. This high morbidity leads to significant disruptions in livestock production due to decreased feeding, lameness, and weight loss. The disease also poses a more severe threat to young animals, where mortality can be significant (Zewdie et al., 2023).

Important livestock species affected by FMD include cattle, pigs, sheep, and goats, all of which are susceptible to infection. Among these, cattle are often considered the maintenance host in most regions, playing a key role in sustaining and spreading the virus within herds and to other species. Pigs, on the other hand, can act as amplifiers of the virus due to the large quantities they shed. While sheep and goats tend to exhibit milder or asymptomatic infections, they can still contribute to the silent spread of FMD, complicating control efforts (Chakraborty, 2014; Metwally et al., 2021). Foot-and-mouth disease virus is commonly transmitted through direct animal-to-animal contact. Infected animals shed the virus in all bodily secretions and excretions, including saliva, urine, feces, milk, and even aerosols. This widespread viral shedding facilitates rapid transmission within herds, as animals can easily become infected through contact with contaminated surfaces, feed, water, or through inhalation of viral particles. The highly contagious nature of FMDV underscores the need for stringent biosecurity measures to prevent the spread of the virus during outbreaks (Raoof, 2024).

Foot-and-mouth disease (FMD) is endemic in Nigeria, with several serotypes of the virus circulating in the region. Specifically, serotypes O, A, SAT 1, and SAT 2 have been isolated and characterized. This endemicity presents ongoing challenges for the livestock industry and disease control in Nigeria, with significant implications for animal health and agricultural productivity (Begovoeva et al., 2023). In Nigeria, various breeds of sheep and goats are distributed across different ecological regions, with the majority being reared under semi-intensive to extensive management systems. These systems, where animals graze freely or interact with minimal confinement, heighten the risk of FMD transmission. Small ruminants harboring the virus asymptomatically act as reservoirs, transmitting FMD to cattle and other susceptible species. This silent transmission helps maintain the infection cycle, contributing to periodic outbreaks of the disease (Begovoeva et al., 2023).

Serological tests play a crucial role in detecting antibodies to FMDV, making them indispensable in various aspects of disease management. They are particularly valuable for sero-surveillance, certifying animals for export, and monitoring immunity levels after vaccination. Specifically, tests for the non-structural protein (NSP) of the virus provide evidence of previous or current viral replication in the host, regardless of vaccination status. An NSP-ELISA is particularly useful, as it can detect antibodies to the polyprotein 3ABC of FMDV, serving as an indicator of past or active infection (Longjam et al., 2011; Zia et al., 2022).

In Benue State, Nigeria, there is a notable gap in research concerning the seroprevalence of FMD in small ruminants and its associated spatial distribution. Understanding the seroprevalence in small ruminants could provide crucial insights into the epidemiology of FMD in Benue State, guiding effective control and prevention measures. Controlling FMD is critical not only for maintaining the health and welfare of livestock but also for safeguarding food security, as outbreaks can severely disrupt the supply chain of meat, milk, and other animal products. Furthermore, mapping the spatial distribution of FMD in the region would enhance the understanding of the disease's transmission patterns, helping policymakers and veterinarians design targeted interventions.

Materials and Method

Study area

Benue State is located in Nigeria's Middle Belt region, positioned between latitudes 6.5° and 8.5° North and longitudes 7.47° and 10° East. Its central location within the country makes it geographically distinct, as it shares borders with six neighboring states: Nassarawa to the north, Taraba to the east, Kogi and Enugu to the west, and Ebonyi and Cross River to the south. Additionally, Benue State has an international boundary with the Republic of Cameroon to the southeast (Ministry of Finance and Economic Planning, n.d.). The state is divided into three senatorial districts - Zone A, B, and C. For this study, the local government areas (LGAs) of Katsina-Ala, Makurdi, and Otukpo were chosen due to their significance as major entry points for trade animals into the respective zones of the state from Northern Nigeria, classifying these locations as high-risk zones for disease transmission (Ogiji, 2018).

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Study design

This epidemiological sero-survey employed a cross-sectional approach to investigate the presence of antibodies against the 3ABC polyprotein of FMDV in small ruminants within Benue State, Nigeria. The study targeted both apparently healthy and clinically ill sheep and goats, with samples collected from abattoirs, markets, and individual farms across selected local government areas in the state's three senatorial districts. A multistage sampling method was utilized, combining simple random selection and judgmental sampling, which focused on animals exhibiting suspected clinical signs.

Sample size

The sample size for this study was calculated using the formula outlined by Thrustfield (1995). A prevalence rate of 27.84%, as reported by Lazarus et al., (2012) in some border states of Nigeria, was applied to determine the sample size with a precision of 5% and a 95% confidence interval. This calculation yielded a minimum required sample size of 309 animals. A total of 402 animals (305 goats, and 97 sheep) were sampled from the selected LGAs, and the ratio of sheep to goats was estimated at 1:3 based on the population of sheep and goats in Nigeria reported by Opasina and Davide-West (1985).

$$n = \frac{Z^2 \times Pexp \times (1 - Pexp)}{d \times d}$$

where: n = required sample size;

Z = z-score

Pexp = expected prevalence;

d = desired absolute precision.

Pexp = 27.84%

d = 5%

Z value for 95% confidence level = 1.96

$$n = \frac{1.96^2 \times Pexp \times (1 - Pexp)}{d \times d}$$

$$n = \frac{1.96^2 \times 0.2784 \times (1 - 0.2784)}{0.05 \times 0.05} = 309$$

Sample collection, processing and transportation

Blood samples were collected from sheep and goats with no history of FMD vaccination. The samples were aseptically drawn from the jugular vein of live animals using a 21G needle attached to a 5 mL syringe, then transferred into 10 mL sterile plain test tubes, which were clearly labeled. Approximately 3-5 mL of blood was collected from each animal, and the owners' consent was always obtained prior to sampling. After collection, the blood samples were allowed to clot, and the serum was carefully harvested. The sera were then transferred into 5 mL sterile tubes, properly labeled, and transported on ice in a cooler to the Foot and Mouth Disease Laboratory at the National Veterinary Research Institute (NVRI), Vom. Upon arrival, the samples were stored at -20°C until further laboratory analysis.

Laboratory analysis

3ABC-TRAPPING ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Foot and mouth disease virus 3ABC-trapping ELISA (produced by IZSLER Biotechnology Laboratory, Brescia, Italy) was used to detect the antibodies to NSP 3ABC of FMDV in the serum samples, and procedures were carried out according to manufacturer's instructions. All solutions and reagents were equilibrated at room temperature before use. Pre-dilutions of the test sera and control sera were performed on a carrier microplate by distributing 95 µL of the ELISA buffer in each well. Thereafter, 5 µL of the test sera and control sera were added into the appropriate wells. After dilution of the test sera and control sera, the sealed and sensitized ELISA microplate were open and 40 µL of

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ELISA buffer was distributed in each well. Using an eight tips multichannel micropipette, 10 µL of each sample and control sera from the carrier plate was transferred into two appropriate columns of the ELISA plate. The tips of the multichannel micropipette were changed for each column of the carrier plate. Post addition of the test and control sera, the plates were gently shaken to properly mix and thereafter covered and incubated for 1 hour at room temperature. After incubation, the wells were emptied and tapped hard to remove all remaining residual fluid. The wells were filled with 200 µL each of the washing solution and incubated for three minutes at room temperature. After incubation, the wells were emptied and the procedure was repeated two more times to bring a total of three washing cycles. Post washing, 50 µL of the appropriate diluted anti-ruminant IgG Horse Radish Peroxidase (HRPO) conjugate was added to the wells, covered and incubated for 1 hour at room temperature. Thereafter, washing cycles were performed as initially described leaving the last one for 5 minutes. The next step involved the distribution of 50 µL/well of the substrate/chromogen solution (equilibrated at room temperature) to all wells, and the plates were covered and left at room temperature for 20 minutes in the dark. Timing began when the first well was filled. After 20 minutes of incubation in the dark, the reaction was stopped by adding 50 µL/well of the stop solution following the same order used for adding the substrate solution. Contents of the plate were rocked gently to mix prior to reading of the results.

Reading of results

Immediately the reaction was stopped, the Optical Density (OD) of each well was read at 450 nm wavelength using the Multiskan® ELISA reader (Thermo Scientific, USA). The OD of each test serum and control serum in the wells without antigen was subtracted from the OD of the wells containing antigen to give the net OD. The percentage positivity of each test serum was calculated using the formula below.

$$\%\ positivity = \frac{net\ OD\ value\ of\ test\ serum}{net\ OD\ value\ of\ positive\ control\ serum} \times 100$$

Test sera with results <10% were considered negative, and those with results ≥10% were considered positive.

Data analysis and visualization

Descriptive statistics was used to represent percentage seropositivity for small ruminant species and geographical locations. Confidence Interval (CI) for proportion was calculated by the modified Wald method using GraphPad QuickCalcs at 95% CI. The Chi-Square Test for Independence was performed using Microsoft Excel to determine if seropositivity was significantly associated with geographical locations, sub-locations, and species.

Results

The overall seroprevalence of FMDV in small ruminants in Benue State, Nigeria, was 15.92% (Table I), with goats showing a seroprevalence of 14.75% and sheep at 19.59% (Table II). In Katsina-Ala (Zone A) and Makurdi (Zone B), the seroprevalence rates were 14.71% and 14.72% respectively and Otukpo (Zone C) had the highest rate at 19.42% (Table I). The spatial distribution of seroprevalence across sub-localities of representative LGAs in Zones of the state is detailed in Table I.

Zones and LGAs	Sub-locations	Sample Size	Seropositive	Prevalence (%)	95% CI	*df*	$\chi 2$	*p-value*	****
KATSINA-ALA (Zone A)	Katsina-Ala township	55	13	23.64	14.24 - 36.47	2	81.864	0.01669	Yes
	Gboroya	25	2	8.00	1.09 - 26.1				
	Abaver village	22	0	0.00	0 - 17.55				
	Sub-total	**102**	**15**	14.71	9 - 22.97				
MAKURDI (Zone B)	North Bank	76	4	5.26	1.67 - 13.16	2	212.911	2.38E+00	Yes
	Wurukum	67	7	10.45	4.87 - 20.32				
	Wadata	54	18	33.33	22.19 - 46.69				
	Sub-total	**197**	**29**	14.72	10.40 - 20.39				
OTUKPO (Zone C)	Otukpo township	52	11	21.15	12.08 - 34.20	1	0.2024	0.6528	No
	Hausa community	51	9	17.65	9.34 - 30.48				
	Sub-total	**103**	**20**	19.42	12.86 - 28.17				
Grand total		**402**	**64**	15.92	12.65 - 19.83				

CI: Confidence Interval for Proportion, df: Degrees of freedom, $\chi 2$: Chi-Square value, **** Comment on statistical significance

Table I. Spatial seroprevalence of FMDV in small ruminants in Benue State, Nigeria.

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Species	Sample Size	Seropositive	Prevalence (%)	95% CI	*df*	χ2	*p-value*	****
Caprine	305	45	14.75	11.19 - 19.20	1	12.845	0.2571	No
Ovine	97	19	19.59	12.83 - 28.66				
Total	402	64	15.92	12.65 - 19.83				

CI: Confidence Interval for Proportion, df: Degrees of freedom, χ2: Chi-Square value, **** Comment on statistical significance

Table II. Species-based seroprevalence of FMDV in small ruminants in Benue State, Nigeria.

Discussion

The seroprevalence of FMDV in small ruminants in Benue State, Nigeria, was found to be 15.92%, with 14.75% in goats and 19.59% in sheep. These findings contribute to the growing body of evidence regarding the prevalence of FMDV in small ruminants across various regions of Nigeria while also underscoring certain regional differences and similarities when compared with previous studies.

In comparison to the study by Lazarus et al., (2012), who reported a seroprevalence of 27.84% in small ruminants in Bauchi State, the overall seroprevalence of our study is lower. We attribute this to differences in livestock management practices, animal movement patterns, and proximity to major reservoirs of FMDV, such as cattle and wildlife. The specific prevalence rates of 14.75% for goats and 19.59% for sheep, though not statistically significant, indicate a possible variation in susceptibility between species, suggesting that sheep may be more susceptible to FMDV infection than goats in this region. The observed prevalence in sheep (19.59%) aligns closely with findings by Olabode et al., (2019), who reported 16.0% seroprevalence in sheep slaughtered at the Gwagwalada abattoir in Abuja.

Spatial seroprevalence based on sub-localities showed statistically significant association with seropositivity. Statistically significant differences were observed between the sub-localities of Makurdi and Katsina-Ala, with Wadata and Katsina-Ala township accounting for this difference. This finding is consistent with the fact that many northern states are connected to Benue State through Makurdi, resulting in a significant influx of trade animals entering the state via this LGA. Otukpo ranked highest in prevalence among the three LGAs, likely due to the supply of trade animals originating from both Makurdi and Katsina-Ala. When comparing our findings to the larger-scale study by Begovoeva et al., (2023), which reported a lower overall seroprevalence of 10.2% in small ruminants in Northern Nigeria, it becomes evident that the FMDV prevalence in Benue State is slightly higher than the northern region's average. The state-level prevalence estimates provided by Begovoeva et al., (2023) reveal significant variability, with 17.3% in Kaduna, 6.9% in Bauchi, and 3.6% in Plateau State. Given that Kaduna shares trade routes with Benue through Makurdi and Otukpo, our finding of 15.92% fits more closely with the higher end of the spectrum seen in Kaduna.

In contrast, significant differences in FMDV exposure are observed when expanding the comparison to large ruminants, such as cattle. Studies by Wungak et al., (2016) reported much higher seroprevalence rates in cattle, with 85.4% in Niger State and 54.2% in Plateau State. The high seroprevalence in these states, which serve as both source and transit zones for trade animals entering Benue, shows that cattle are major reservoirs of FMDV, amplifying transmission risks through their movement during grazing, market activities, and migration. Similarly,1 Atuman et al., (2020) reported a 65.7% seroprevalence in cattle in Bauchi State, reinforcing the notion that cattle populations face a higher risk of FMDV exposure compared to small ruminants.

The findings from our study also differ significantly from those observed in camels, as reported by Ularamu et al., (2015), where the seroprevalence of FMDV was 10.83%. The lower prevalence in camels may be attributed to their distinct grazing habits and limited interaction with small ruminants in mixed farming systems, as well as differences in herd management practices. Lastly, the seroprevalence in wildlife populations, as reported by Atuman et al., (2020) in Bauchi State (24.5%), highlights the potential role of wildlife in maintaining and spreading FMDV. The movement of wildlife across trade routes may facilitate the spillover of FMDV to domestic livestock, particularly in areas where wildlife and livestock cohabit or interact.

Overall, our study suggests that small ruminants in Benue State are significantly exposed to FMDV, with prevalence rates comparable to some regions and lower than others, especially when compared to large ruminants like cattle. The findings underscore the necessity for region-specific control strategies that take into account the dynamics of animal movement and trade routes. Furthermore, further investigations into the drivers of FMDV transmission between species and across different regions in Nigeria are essential. Implementing effective surveillance and biosecurity measures, particularly along high-risk trade routes, will be crucial in mitigating the impact of FMDV and ensuring the

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economic sustainability of livestock farming in the region.

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