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Serological Study of Middle East Respiratory Syndrome Corona Virus (MERS-CoV) Strains Circulating Among Camels and Their Handlers in Some Parts of North-Eastern Nigeria

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ABSTRACT

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is increasingly recognized as a significant emerging zoonotic disease of global health concern, affecting camel handlers due to its high mortality rates. However, there is limited data available on this issue in the study areas. This study aimed to assess the relationship between socio-demographic factors, seasonal variation (wet and dry seasons), and the prevalence of MERS-CoV infection among the population in North-eastern Nigeria, including Borno, Yobe, and Bauchi States. A total of 780 camel handlers were recruited, with 260 participants from each state. In addition, 120 dromedary camels (40 from each state) were tested for MERS-CoV. Blood samples were screened for MERS-CoV IgM antibodies using an ELISA kit (Axion Diagn, Germany) as per the manufacturer's instructions. A structured questionnaire was employed to collect socio-demographic data, and the relationship between MERS-CoV sero-prevalence (IgM antibodies) and socio-demographic characteristics /seasonal variation was analysed using SPSS software (version 23.0). The overall sero-prevalence of MERS-CoV infection among camel handlers was 8.1% (63/780), while in camels it was 43.8% (135/240), with higher rates during the dry season (6.8% in handlers and 58.3% in camels), which was statistically significant (P<0.05). MERS-CoV infection was more common in males (7.7%, 60/780) compared to females (0.4%, 3/780), particularly in the 40-49 age group (3.6%, 28/780), and was most prevalent among camel handlers from Borno State (3.5%, 27/780). Factors such as camel rearing, low education levels, and lack of awareness significantly contributed (P<0.05) to the high infection rate. Therefore, public awareness programs focusing on MERS-CoV transmission and preventive measures, especially among camel and animal handlers, are essential to limit the spread of the disease and reduce its associated morbidity and mortality.

Keywords: ***Camel, dromedary, MERS-CoV, sero-prevalence***.

INTRODUCTION

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a newly recognized zoonotic virus (Degnah *et al*., 2020; WHO, 2024). The initial case of MERS-CoV was identified in a hospitalized male in Jeddah, Saudi Arabia (Butler, 2012; Zaki *et al*., 2012; WHO, 2022), with compelling evidence indicating that dromedary camels act as the reservoir host, facilitating the transmission of the virus to humans (FAO-OIE-WHO MERS Technical Working Group, 2018). The virus has persistently posed significant public health, security, and economic challenges worldwide. So far, more than 2,500 instances of human illness have been documented by the World Health Organization (WHO), with cases disseminated to multiple global regions (WHO, 2022). The virus proliferates extensively in dromedary camels without inducing substantial sickness in them (Dighe *et al.,* 2019). MERS-CoV is spread to people via

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animals and by human-to-human contact (Ithete *et al*., 2013; Zhu *et al.,* 2020). Research (Ge *et al*., 2013) indicates that MERS-CoV in bats is capable of infecting humans. Human contact with bats or their excretions can occasionally facilitate the transmission of MERS-CoV to humans (Woo *et al*., 2013; WHO, 2022).

MERS-CoV is a species of coronaviruses belonging to the new lineage C beta coronavirus, which causes severe and acute respiratory disease in humans. Formerly known as "novel human coronavirus," it has now been recognized as a major human pathogen. In 2015, the World Health Organization (WHO) documented 1,618 laboratory-confirmed instances of MERS, leading to 579 fatalities (WHO, 2015). This recently identified beta coronavirus from clade C has been associated with severe respiratory illness in humans, especially in regions of the Middle East (ECDC, 2015). The case fatality rate for human infection with MERS-CoV is around 35% (World Health Organization, 2018). Following spillover occurrences from animals to people, human-to-human transmission is often inefficient and usually transpires mainly in tight, unprotected contact settings, such as hospitals (Breban *et al*., 2013; Cauchemez *et al*., 2013; CDC, 2024). Phylogenetic analysis of viral genomes from dromedaries and humans indicates that numerous camel-to-human spillover events have probably occurred since 2012 (Dudas *et al*., 2018).

MERS-CoV represents a considerable zoonotic risk to global public health, chiefly because of its elevated fatality rate associated with the severe acute respiratory syndrome it induces (Mohd *et al*., 2016). The absence of efficient antiviral therapies and preventive vaccinations exacerbates the issue (Al Kahlout *et al*., 2019). Since its emergence, the virus has disseminated throughout multiple nations in the Arabian Peninsula, infecting nearly one thousand individuals with a significant case fatality rate (WHO, 2019). In severe instances, particularly in immunocompromised persons and those with chronic problems such as diabetes or pulmonary ailments, the infection may result in life-threatening sickness, frequently necessitating intense medical intervention and leading to elevated fatality rates (Chan *et al.,* 2015). This study seeks to ascertain the seroprevalence of MERS-CoV antibodies in camels and their handlers in Northeastern Nigeria.

MATERIALS AND METHODS

Study Area

The research was carried out in the Northeast geopolitical zone of Nigeria, comprising six states: Adamawa, Bauchi, Borno, Gombe, Taraba, and Yobe. This region encompasses almost one-third of the nation's land area and has a population of 28,182,725, constituting 14.01% of Nigeria's overall population (NPC, 2019). The investigation focused on three states: Borno, Yobe, and Bauchi, chosen for their superior camel numbers relative to other regions. Borno State is located between latitudes 10°N and 13°N and longitudes 12°E and 15°E, encompassing an area of approximately 69,436 square kilometers, with an estimated population of 4.2 million (NPC, 2006). It shares international boundaries with Niger, Chad, and Cameroon, enabling the cross-border movement of livestock among these adjacent nations (Bourn et al., 1994). Borno shares its northern boundary with Niger, its eastern boundary with Cameroon, and its northeastern boundary with Chad (Figure 1).

Bauchi State is situated between latitudes 8°N and 11°N and longitudes 11.50°E and 13.50°E. It is among Nigeria's largest states, encompassing approximately 36,917 square kilometers. Yobe State is located in a latitude of 12°N and a longitude of 13°E, with a

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geographical area of 45,502 square kilometers. The state generally endures a dry and hot climate for most of the year, with the exception of the southern part, which exhibits a milder climate (Frederick *et al*., 2006).

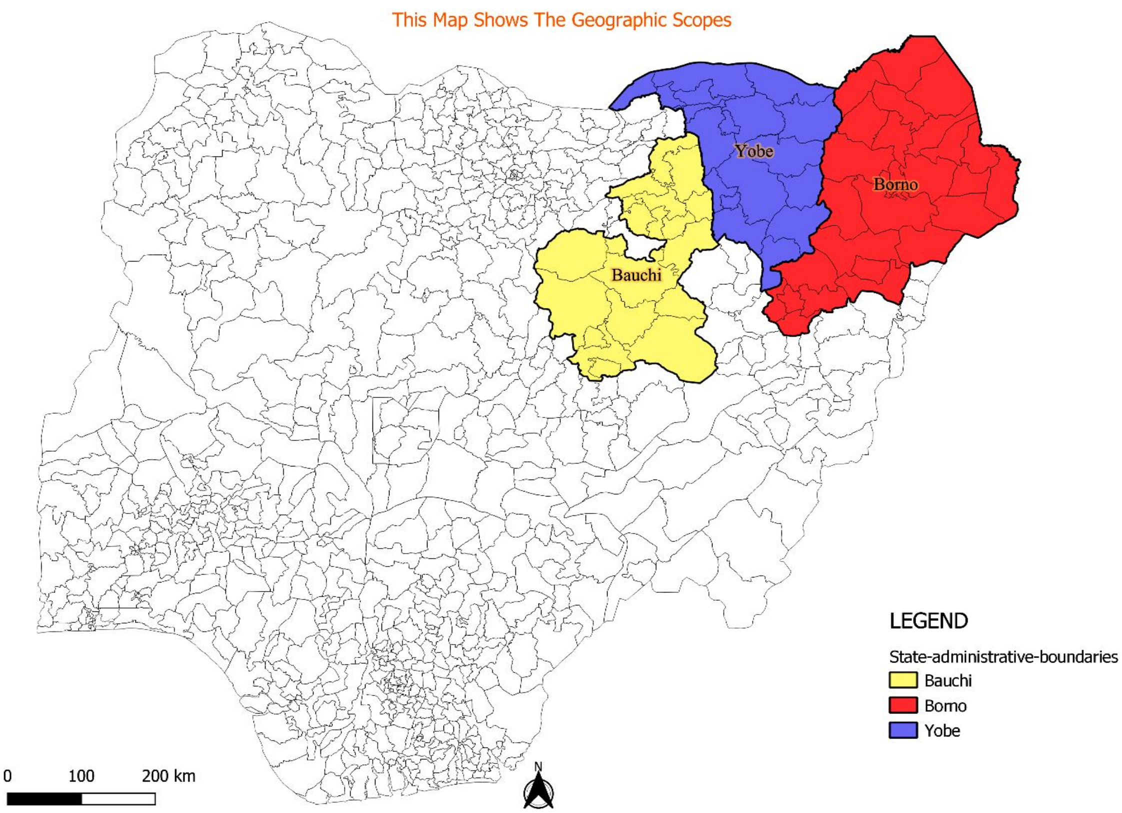

Figure 1: Map of the study area.

Study Population

The study population comprised of the camels and camel herds as well as their handlers in the North-Eastern Nigeria with permission and ethical approval from respective states ministry of Health of the concerned North-Eastern States.

The handlers were the group of people living together (having lived together or interacted with the camels for a certain period, at least one month) through one manner of contact or the other. University lecturers who were not exposed to camels in one way or the other were referred to as the control group.

Study Design

The study utilized a cross-sectional and descriptive design to assess the prevalence of MERS-CoV antibodies circulating among camels and their handlers. It also aimed to explore the relationship between MERS-CoV infection and various risk factors, including socio-demographic factors, in the study area.

Determination of Sample Size

The sample size for camel handlers was determined based on the seroprevalence rate of 19.4% reported by Gaddafi *et al*. (2020) in Nigeria and seroprevalence rate of 31% reported by Islam *et al* ,2023 in Bangladesh for camels globally, the sample size formula as proposed by Lwanga and Lemeshaw (1991)

$n = \frac{z^2pq}{d^2}$ (Lwanga, 1991)

Where;

n = Sample size

z = Standard Normal distribution at 95% Confidence Interval = 1.96

p = Prevalence of 19.4% (0.194)

q = 1-p = (1-0.194) = 0.806

d = allowable error (5%) = 0.05

Substituting the values in the formula:

$$n = \frac{1.96^2 \text{x } 0.194(0.806)}{0.05^2}$$

$$n = \frac{0.601}{0.0025} = 240.4/State$$

P= 31% (0.31)

(1 - 0.31)= 0.69

n= 1.96^2x0.31 (0.69)

0.0025

Thus a total of 780 camel handlers and 120 camels were the figures obtained; that is, 260 camel handlers per state and 40 camels per each state respectively were examined during the study.

Ethical Clearance

Permission and ethical approval was sought to conduct this research from respective states ministry of Health of the concerned North-Eastern States (Appendix I).

Inclusion/Exclusion Criteria

Blood samples were collected from both sexes of camels. The focus was on camels living around areas in North-East Nigeria only and the population of their handlers who had consented to participate in the study. University lecturers who had no any contact with the dromedary camels were also included as control.

Consent of Study Population (Camel handlers)

A designed consent form was administered to camel handlers who have accepted to participate in the study with full confidentiality (Appendix II).

Sampling Techniques

Simple random sampling technique was adopted by the study. The study was divided into three zones based on the existing centres where camel rearing is prevalent and existing slaughtering point in the selected zones. The samples consist of a population of dromedaries in North-eastern Nigeria and all individuals who in one way or the other come into contact with camel or involve in slaughtering the camels within the selected zones that covered Borno, Yobe and Bauchi States. Thus, selection of respondents in the entire zone was based on wilful acceptance. University lecturers in the states that did not have any contact with the dromedaries were regarded as the controls.

Data Collection Using Questionnaires

A number of adopted validated questionnaires were administered to the subjects. The questionnaires were made up of two segments: Section A consists of personal information on

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socio-demographic status of the respondents while section B consists of MERS-CoV related information (Appendix IV)

Sample Collection, Preparation, Transportation and Storage

Blood samples (10mls) were collected from camels during slaughter at the abattoirs and 5mls obtained from their handlers were introduced into a separate clean, dry vacutainer tubes and labelled accordingly. Nasal swabs of the camels were also collected. Specimens were accompanied with levels of basic information. Sample Transportation was according to WHO (2016) standard operating procedures for transport of biological (human/animal) specimens which include among others proper packaging using leak proof containers and bags, appropriate labels with biohazard symbol, appropriate temperatures with the use of coolers and dry ice, clear labels of sample names and identification numbers. The blood samples were centrifuged at 1,500 rpm for 5 minutes, after which the serum was meticulously transferred into sterile, dry cryo-tubes utilizing sterile Pasteur pipettes. The sera were thereafter preserved at -80°C till analysis was conducted.

Determination of Seroprevalence

MERS-CoV Serological Assay

The MERS-CoV serological assay was conducted according to the procedure described by Gaddafi *et al*. (2020). Serum samples from camels and their handlers, obtained from Borno, Bauchi, and Yobe, were evaluated with the recombinant S Protein Enzyme-Linked Immunosorbent Assay (ELISA). The examination was executed in accordance with the manufacturer's specifications, as outlined below.

Principle of ELISA

The sera to be tested were added to MERS-CoV antigen-coated microplates and incubated for a short period. If specific antibodies were present, they bound to the antigen. To detect the antigen-antibody reaction, the ELISA method utilized anti-HGG (an antibody that reacts with any human immunoglobulin), chemically linked to an enzyme label, such as peroxidase. This enzyme-bound anti-HGG attached to the test antibody. Any excess labeled anti-HGG was washed away, and chromogen was added. Chromogen is a colourless substrate that, when acted upon by an enzyme like peroxidase, produces a coloured end product. The colour change was then measured quantitatively using optical density (OD) values generated through spectrophotometry, which directly correlates with the amount of antibody bound (Nester *et al*., 2004).

Antibody ELISA Assay Procedure for Mers-CoV

The plates for the MERS-CoV antibody ELISA (DRG® MERS-CoV IgM (EIA)-1797), produced by Axiom Diagnostics Germany (DRG-Intl USA), Batch No 70831, were pre-coated with purified MERS-CoV antigen. Diluted sera or swab samples were added to the wells, where any MERS-CoV IgM-specific antibodies present would bind to the antigen. Following the cleaning of the wells to eliminate any unbound samples, a solution of human IgM antibodies conjugated with horseradish peroxidase (HRP) was introduced and incubated at 37°C for 30 minutes. After a further washing step to eliminate unbound conjugate, a TMB reagent solution was added to the microwells. The catalytic activity of the enzyme conjugate was halted after 20 minutes. The color intensity generated was directly related to the concentration of virus-specific IgM antibodies in the sample. The results were analyzed using a microwell reader and

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concurrently compared with calibrators and controls at a wavelength of 450 nm.

Detection of Mers –CoV IgM by ELISA

All stored samples and reagents kits were brought to room temperature (18 – 25^{o}C). The 100ml buffer provided was diluted with 900ml distilled water to make 1000ml. The microtitre wells were labelled (reagents standard wells, two negative control wells two positive control wells and the rest sample wells). Five microliters (5 µl) of each sera / camel swabs were diluted with 100µl of the sample diluents. One hundred microliters (100µl) of the diluted sera and controls were dispensed into the sample and control wells respectively. The plate was sealed with a plate sealer and incubated for 20 minutes at ambient temperature. The plate had three washes with wash buffer, each involving a 20-second swirling, followed by blot drying on absorbent paper. Subsequently, 100 µl of conjugate was dispensed into each well. The plate was sealed with a plate sealer and incubated at 37°C for 20 minutes. Subsequent to incubation, the plate underwent four washes with the wash buffer, each accompanied by swirling for 20 seconds, followed by blot drying on absorbent paper. Subsequently, 100 µl of Tetra-Methyl Benzidine (TMB) substrate was introduced to each well and incubated at 37°C for 10 minutes. The reaction was terminated by the addition of 100 µl of stop solution to each well, and the plate's bottom was wiped clean. The microplate reader (GF-M 3000, Model SNON E-Max Molecular Device) was calibrated at 450 nm using the blank well, and the absorbance (A) of each well was recorded at the same wavelength within 10 minutes.

Interpretation of ELISA Results

Cut-off calibrator IgM index = 1.0

Mers- CoV - Index less than 0.90 is negative

1. "Cut-off Calibrator O.D = 0.781, 0.757 $X_c = 0.749$
2. Negative Control O.D = 0.055, 0.071 $X_n = 0.067$
3. Positive Control O.D = 1.487, 1.545 X_p 1.517
4. Sample O.D = 1.029, 1.046 $X_s = 1.038$"

IgG – cut off value is 0.151, any number less than 0.151 is negative, any number greater than 0.151 is positive.

Data Analysis

Data obtained were analysed using SPSS version 23.0 While, the prevalence of MERS–CoV antibodies among camels and their handlers was analysed in frequency and percentages. Chi-square analysis was used, for the means of the prevalence of the MERS-CoV antibodies that were significant at 5% level.

RESULTS

Socio-Demographic Characteristics of the Subjects Surveyed (n=780)

The results for the socio-demographic status of the respondents are displayed in Table 1. The results indicated that 73.46% (n=573) of the respondents were males while 26.54% (n=207) of them were females. Based on the results obtained, the highest number of respondents

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(31.15%) was between the ages of 40-49 years followed by those above 50 years (23.07%). When marital status was considered, 75.51% of the respondents were married and 50.89% of them were camel handlers. With respect to educational qualification, 60.89% of the respondents did not have formal education with 9.10% having tertiary education.

Table 1: Socio-Demographic Characteristics of the subjects surveyed (n=780).

Variables	Response	Frequency (n= 780)	Percentage (%)
Gender	Male	573	73.46
	Female	207	26.54
	Total	**780**	**100**
Age (in years)	10-19	60	7.69
	20-29	131	16.79
	30-39	166	21.28
	40-49	243	31.15
	≥50	180	23.07
	Total	**780**	**100.0**
Marital Status	Married	589	75.51
	Single	160	20.51
	Divorced	5	0.64
	Widow/Widower	26	3.33
	Total	**780**	**100**
Occupation	Salesman	107	13.72
	Handler	397	50.89
	Butcher	218	27.95
	Veterinarian	28	3.59
	Lecturer	30	3.85
	Total	**780**	**100**
Educational Status	Non Formal	475	60.89
	Primary	128	16.41
	Secondary	106	13.59
	Tertiary	71	9.11
	Total	**780**	**100**

Values are presented as Percentage, Using Chi-square test for independence, n= 780
Values with $p<0.05$ indicates significant association.
p value for the above table is 0.000.

Prevalence of MERS-CoV Antibodies in Camels/Handlers from North-East Nigeria

Table 2 presents the sero-prevalence data of MERS-CoV antibodies in dromedary camels from northeastern Nigeria. The results reveal that in the dry season, the highest frequency of MERS-CoV antibodies was detected in camels from Borno State (31 camels, 25.83%), whilst the lowest prevalence was noted in camels from Bauchi State (17 camels, 14.17%). A comparable pattern was noted in the wet season, with the greatest quantity of camels exhibiting MERS-CoV antibodies in Borno State (16 camels, 13.33%) and the least in Bauchi State (8 camels, 6.67%). The prevalence of MERS-CoV antibodies was greater in the dry season (70 camels, 58.33%) than in the rainy season (35 camels, 29.17%). Throughout both seasons, 87.5% of dromedary camels exhibiting MERS-CoV antibodies were situated in the northeastern region.

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Table 2: Prevalence of MERS-CoV antibodies among camels in Nigeria.

Season	Location	Total No. Tested	No (%) Positive IgM	No (%) Negative
Dry	Borno	40	31 (25.83)	9 (7.50)
	Yobe	40	22 (18.33)	18 (15.00)
	Bauchi	40	17 (14.17)	23 (19.17)
	Overall Total	**120**	**70 (58.33)**	**50 (41.67)**
Wet	Borno	40	16 (13.33)	24 (20.00)
	Yobe	40	11 (9.17)	29 (24.17)
	Bauchi	40	8 (6.67)	32 (26.67)
	Overall Total (%)	**120**	**35 (29.17)**	**85 (70.83)**

Values are presented as Percentage, Using Chi square test for independence, n= 240.
Values with $p<0.05$ indicates significant association.

The results for the Sero-Prevalence of MERS-CoV Antibodies among Camel Handlers from North-eastern Nigeria

The seroprevalence findings of MERS-CoV antibodies among camel handlers in northeastern Nigeria are displayed in Table 3. The results indicate that in the dry season, the highest frequency of MERS-CoV antibodies was found among camel handlers in Borno State (33 handlers, 8.46%), whilst the lowest prevalence was noted in Bauchi State (31 handlers, 25.83%). Comparable trends were seen throughout the three investigated locations during the wet season. The incidence of MERS-CoV antibodies was greater in the dry season (53 handlers, 13.59%) than in the rainy season (10 handlers, 2.56%). The overall sero-positivity among camel handlers over both seasons was 16.15%.

Table 3: Prevalence of MERS-Cov antibodies among camel Handlers in North East Nigeria.

Season	Location	Total No. Tested	No (%) Positive IgM	No (%) Negative
Dry	Borno	130	33 (8.46)	97 (24.87)
	Yobe	130	12 (3.08)	118 (30.26)
	Bauchi	130	8 (2.05)	122 (31.28)
	Total	**390 (100%)**	**53 (13.59)**	**337 (86.41)**
Wet	Borno	130	6 (1.54)	124 (31.79)
	Yobe	130	3 (0.77)	127 (32.56)
	Bauchi	130	1 (0.26)	129 (33.08)
	Overall Total	**390 (100%)**	**10 (2.56)**	**380 (97.44)**

Values are presented as Percentage, Using Chi square test for independence, n= 780.
Values with $p<0.05$ indicates significant association.

Distribution of MERS-CoV Antibodies Based on Gender among the Subjects in North-eastern Nigeria

The findings on the distribution of MERS-CoV antibodies by gender among participants in North-eastern Nigeria are illustrated in Table 4. The highest prevalence of MERS-CoV antibodies was observed in men, with rates of 14.23%, 5.39%, and 3.46% in Borno, Yobe, and Bauchi States, respectively. In contrast, the prevalence rates for females were 0.77% in Borno and 0.39% in Yobe state, while Bauchi reported no positive cases.

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Table 4: Prevalence of MERS-CoV Antibodies based on Gender of camel handlers in North-East Nigeria.

No examined:780				
State	**Gender**	**Total No (%) Examined**	**No (%) Positive IgM**	**No (%) Negative**
Borno	Males	176 (67.69)	37 (14.23)	139 (53.46)
	Female	84 (32.31)	2 (0.77)	82 (31.54)
	Total	**260 (100.00)**	**39 (15.00)**	**221 (85.00)**
Yobe	Males	225 (86.54)	14 (5.39)	211 (81.15)
	Female	35 (13.46)	1 (0.39)	34 (13.08)
	Total	**260 (100)**	**15 (5.77)**	**245 (94.23%)**
Bauchi	Males	172 (66.15)	9 (3.46)	163 (62.69)
	Female	88 (33.85)	0 (0.00)	88 (33.85)
	Overall Total	**260 (100%)**	**9 (3.46%)**	**251 (96.54%)**

Values are presented as Percentage, Using Chi square test for independence, n= 780.
Values with $p<0.05$ indicates significant association.

Prevalence of MERS-CoV Antibodies among Camel Handlers in the North-eastern Part of Nigeria Based on Age

Table 5 displays the incidence of MERS-CoV antibodies among camel handlers in northeastern Nigeria, stratified by age. The findings reveal that in Borno State, the highest prevalence (10.77%) was noted among those aged 40-49 years, succeeded by those aged 30-39 years (2.31%). Significantly, no individuals under 30 years of age exhibited positive results for MERS-CoV antibodies in Borno State. Similarly, the highest prevalence of MERS-CoV antibodies (2.69%) in Bauchi was found among people aged 40-49 years old with none below the age of 40 positives for the antibodies. However, in Yobe state, the highest prevalence of the antibodies was found among people aged 50 years to above with a prevalence rate of 3.85%.

Table 5: Prevalence of MERS-CoV Antibodies based on Age of camel handlers in North-East Nigeria.

No examined:780				
State	**Age Range (Years)**	**Total No (%) Examined**	**No (%) Positive IgM**	**No (%) Negative**
	10-19	18 (6.92)	0 (0.00)	18 (6.92)
	20-29	42 (16.15)	0 (0.00)	42 (16.15)
Borno	30-39	45 (17.31)	6 (2.31)	39 (15.00)
	40-49	124(47.69)	28 (10.77)	96 (36.92)
	50-Above	31 (11.92)	5 (1.92)	26 (10.00)
	Total	**260 (100.00%)**	**39 (15.00%)**	**221 (85.00%)**
Yobe	10-19	19 (7.31)	0 (0.00)	19 (7.31)
	20-29	50 (19.23)	0 (0.00)	50 (19.23)
	30-39	63 (24.23)	0 (0.00)	63 (24.23)
	40-49	40 (15.38)	5 (1.92)	35 (13.46)
	50-Above	88 (33.85)	10 (3.85)	78 (30.00)
	Total	**260 (100.00%)**	**15 (5.77%)**	**245 (94.23%)**
Bauchi	10-19	23 (8.85)	0 (0.00)	23 (8.85)
	20-29	39 (15.00)	0 (0.00)	39 (15.00)
	30-39	58 (22.31)	0 (0.00)	58 (22.31)
	40-49	79 (30.38)	7 (2.69)	72 (27.69)
	50-Above	61 (23.46)	2 (0.77)	59 (22.69%)
	Overall Total	**260 (100.00%)**	**9 (3.46)**	**251 (96.54)**

Values are presented as Percentage, Using Chi square test for independence, n= 780

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Values with $p<0.05$ indicates significant association

DISCUSSION

Demography of Camel Handlers

The heightened incidence of MERS-CoV antibodies in individuals aged 40 and above aligns with the findings of Degnaha et al. (2020), who observed comparable results in healthy adults in Saudi Arabia. Nassar *et al.* (2018b) likewise ascribed the disease's prevalence to older age demographics. Sayed *et al.* (2020) found no significant correlation between age and the likelihood of MERS-CoV infection; nevertheless, they noted that individuals in middle adulthood (ages 40-60) had somewhat greater odds of infection compared to younger persons. This slight variation may result from disparities in immunological responses among various age groups. Similarly, Alghamdi *et al*. (2014) indicated that persons aged 45-59 and older exhibited elevated infection rates compared to their younger counterparts. Consequently, the findings of this study corroborate prior research about the influence of age on the epidemiology of MERS-CoV.

The incidence of MERS-CoV antibodies is larger in males than in females, perhaps due to increased exposure to camels. The study's sample of women was restricted due to the historically male-dominated nature of camel handling within the Hausa/Kanuri cultural framework. Therefore, due to the minimal sample size and the restricted involvement of females in camel handling, it is unsurprising that neither of the two women sampled tested positive for MERS-CoV, with all seropositive individuals being male. Numerous research have investigated MERS-CoV in both community and healthcare environments, revealing a higher incidence of infection among men compared to women (Amer *et al.,* 2018). Lee *et al.* (2020) also discovered that males exhibited a higher incidence of diagnosis and fatality than females, with 59.7% of MERS cases being male and 40.3% female. The notable disparity between genders may arise from differing levels of exposure rather than intrinsic biological differences in sensitivity (Gossner et al., 2016).

Chantal *et al*. (2014) ascribed the lack of MERS-CoV infections in Africa to several risk factors, including demographic disparities, regional behaviors, and possible genetic changes in the circulating viral strains. The demographic sero-positivity for MERS-CoV correlates with educational attainment, since those with higher education levels are generally more proactive in safeguarding themselves against disease transmission. Although interaction with camels is acknowledged as a risk factor for MERS-CoV infection, the precise types of camel exposures that lead to MERS-CoV seropositivity remain inadequately elucidated, as indicated by Khudhair et al. (2019).

Prevalence of MERS-CoV Antibodies among Camels in Nigeria

The detection of MERS-CoV antibodies in camels in northeastern Nigeria signifies the virus's circulation and dissemination beyond its initial origination in the Arabian Peninsula. This discovery corresponds with the research of Chantal *et al*. (2014), which documented serological evidence of Middle East respiratory syndrome coronavirus in dromedary camels from Nigeria, Tunisia, and Ethiopia, underscoring the pervasive presence of the disease throughout Africa. The elevated incidence of MERS-CoV antibodies in camels from northeastern Nigeria may be associated with their origins, as numerous camels originated from North African nations, including Tunisia, Egypt, and Algeria, frequently transiting through Niger Republic. The reported prevalence rate of 87.5% in this study aligns with Gaddafi et al. (2020), who observed a prevalence range of 82-96% among camels slaughtered in Kebbi State. This rate, however, is inferior to the 96%, 82%, and 96% prevalence documented in abattoirs in Sokoto, Adamawa, and Borno, Nigeria, respectively (Reusken *et al.,* 2014). The prevalence rate observed in this study is lower than the 95%, 100%, and 100% rates reported in Afar, Somali, and Addis Ababa (Reusken *et al*., 2013).

Epidemiological investigations have demonstrated a direct link between diseased animals and humans (Memish et al., 2013; Paden et al., 2018; Khudhair *et al*., 2019). This discovery resembles the transmission patterns identified in numerous other zoonotic illnesses that persistently provide substantial dangers and elevate morbidity in both human and animal populations (Bashiru and Bahaman, 2018; Common risk factors that render individuals susceptible to the virus encompass occupational exposure, particularly among camel salespeople, butchers, and veterinarians, in addition to direct contact with infected camels, whether through handling live animals or their excrement. Furthermore, persons with chronic disabling diseases, such as diabetes, face an elevated risk (Alraddadi *et al*., 2016; Khudhair *et al*., 2019).

Prevalence of MERS-Cov Antibodies in Camels/Handlers from North-East Nigeria

A fatal zoonotic virus with the potential to incite a worldwide pandemic has been insufficiently recorded in Africa, particularly in Nigeria, possibly due to the restricted human-to-human transmission that predominantly takes place in healthcare environments, as observed by Grant *et al*. (2019) proposed that the rising incidence of asymptomatic MERS-CoV cases in humans could be due to individuals in constant contact with camels acquiring functional immunity to the virus, hence diminishing the severity of the illness. Recent findings from the Animal Production and Health Unit of the FAO suggest that 2,421 cases of MERS-CoV have been identified, resulting in 870 fatalities among humans (FAO, 2019).

Alongside the conventional Middle Eastern nations recognized for elevated MERS-CoV instances, the disease is beginning to manifest in other African countries, including Nigeria (FAO, 2019). The absence of zoonotic MERS-CoV cases in Africa, despite the existence of MERS-CoV–infected dromedaries, presents a perplexing phenomenon. Geographically and genetically diverse strains of the virus may exist in Africa, perhaps exhibiting reduced replication capability in human lung tissues, which could account for the lack of severe MERS infections on the continent (Zhou et al., 2021). Additionally, seasonal fluctuations in disease incidence may potentially account for the absence of MERS-CoV cases in northeastern Nigeria.

Additional risk factors for MERS-CoV infection are milking, cleaning, and ingesting dromedary meat. This corresponds with the observations of Azhar *et al*. (2014) who indicated that dromedaries are vital to numerous populations in Africa, supplying critical resources of milk, meat, and labor in rural regions. The observed prevalence of MERS-CoV antibodies among camel handlers, butchers, and salesmen in this study aligns with the findings of Khudhair *et al*. (2019), which indicated elevated seroprevalence in particular occupational groups, such as camel salesmen and individuals engaged in the transportation of animals or their waste. Prior research on persons with occupational contact to camels has indicated diverse seropositivity frequencies. For example, reduced seropositivity rates were noted among individuals with camel exposure in Qatar (Reusken *et al*., 2015) and among camel herders in Saudi Arabia (Müller *et al.,* 2015).

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A research in Saudi Arabia revealed similar seropositivity rates among camel workers (Alshukairi *et al*., 2018).

The absence of face masks, gloves, and hand washing with detergents and antiseptics has been recognized as a possible vector for viral transmission among camels in Nigeria. This data corroborates Khuzair *et al*. (2019), who observed that the neglect of protective measures, including the use of masks, gloves, and regular hand washing, significantly contributed to the transmission of the virus. Due to the inadequate understanding of camel-to-human transmission of MERS-CoV, the WHO has recommended extensive preventive measures for personnel in slaughterhouses and marketplaces. These measures encompass the use of facial protection where feasible, hand hygiene before and after animal handling, and the sanitation of work attire and footwear to avert contamination of family members (WHO, 2018). Promoting increased compliance with these precautions, particularly among market workers, may mitigate the risk of infection. Nonetheless, the lifetime of MERS-CoV antibodies in the body remains ambiguous (Choe *et al*., 2017), leaving the timing and location of these infections undetermined. Transmission potential exists in the United Arab Emirates beyond merely markets and slaughterhouses. Moreover, it is uncertain whether the illnesses presented with symptoms. Participants were questioned regarding their visits to healthcare providers for respiratory disorders in the last year; however, the reported illnesses did not correlate with seropositivity, suggesting that bacteria other than MERS-CoV may be responsible for the reported respiratory ailments.

The current study's results demonstrate a notable correlation between the elevated incidence of MERS-CoV and seasonal fluctuations. A significant rise in occurrence was noted during the dry season, especially when the harmattan winds occurred from December to February. This corresponds with the findings of Nassar *et al*. (2018), who observed that MERS-CoV infections demonstrate seasonal patterns. The largest incidence of MERS-CoV infections worldwide occurred in June, while the lowest rates were observed from October to January between 2012 and December 2017. This investigation indicated that both camels and their handlers have antibodies specific to MERS-CoV. This finding aligns with the results of Sayed *et al*. (2020), who reported analogous findings in dromedaries and their traders from Upper Egypt. Similar to sero-reactive camels, all seropositive humans appeared healthy, with no history of severe respiratory illness in the 14 days preceding sampling. The presence of particular antibodies in the analyzed camel serum exhibited a significant difference ($P < 0.01$) across different sample locations.

The present investigation revealed discrepancies in the frequency of MERS-CoV within the identical geographical area. This discovery aligns with the findings of Sayed *et al*. (2020), which indicated a greater seropositivity rate of MERS-CoV in Aswan (96.97%) relative to Asyut (35.71%), with no seropositive camels identified in the New Valley Governorate. The disparities in prevalence rates may be ascribed to the lack of infection in seronegative camels or the swift reduction of MERS-CoV antibodies, which can wane within two weeks, as indicated in prior studies (Ali *et al*., 2017).

Furthermore, the heightened seropositivity rate may result from viral transmission among camels during extensive travel, which can induce transport stress and enhance proximity among the animals (Ali *et al*., 2017). Park *et al.* (2018) identified factors associated with MERS-CoV transmission, including extended hospitalizations without isolation, prior hospital admissions or emergency room visits before isolation, mortality rates, and clinical manifestations such as fever and chest X-ray abnormalities (Kim *et al*., 2015 Majumder *et al*., 2017).

The low prevalence rate noted in Bauchi can be ascribed to the transportation of most dromedaries from Niger Republic and Chad without the use of quarantine precautions. This discovery corresponds with the observations of Gaddafi *et al*. (2020) and Kandeil *et al*. (2019), who identified a significant prevalence of MERS-CoV in regions next to the border. This underscores the pressing necessity for enforcing stringent limits on the importation of camels from areas where MERS-CoV is endemic. All camel handlers who tested positive for MERS-CoV in this investigation were asymptomatic and had no history of severe respiratory disease in the 14 days preceding the sampling. Moreover, numerous investigations have recorded the presence of asymptomatic cases associated with MERS-CoV infection in humans; Alshukairi *et al*., 2018).

The ingestion of camel meat, raw milk, and other camel-derived products presents potential concerns for the transmission of MERS-CoV (Sayed *et al*., 2020). Furthermore, Altamimi *et al*. (2020) discerned three principal independent variables correlated with the affirmation of MERS-CoV in Riyadh: age (particularly between 41 and 60 years), gender (predominantly male), and the summer season. Alshukairi *et al.* (2018) documented a significant incidence of MERS-CoV among camel workers, including herders, in Saudi Arabia.

CONCLUSION

The current study identified the presence of specific antibodies against MERS-CoV in camels and their handlers in North East Nigeria. It can thus be concluded from this study that the seropositivity rate of MERS-CoV among camels in North East Nigeria is 87.50%, with the highest prevalence in Borno State at 39.16%. MERS-CoV RNA was detected in nasal swabs from 41.67% of the camels during the dry season, with antibody titres ranging from 180 to 320. The seroprevalence of MERS-CoV antibodies among camel handlers in North East Nigeria is 16.15%, with the highest prevalence also in Borno State. Also, significant risk factors for MERS-CoV seropositivity include occupational exposure (salesmen, handlers, and butchers), educational background (particularly a lack of formal education), seasonal variation (higher rates in the dry season), and geographic location. Notably, the prevalence of MERS-CoV antibodies is greater during the dry season compared to the wet season.

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