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SEROPREVALENCE OF *Yersinia pestis* INFECTION AMONG PATIENTS ATTENDING LOCAL HERBAL CENTRES IN ABAKALIKI DISTRICT, EBONYI STATE

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ABSTRACT

Yersinia pestis is a gram-negative coccobacillus bacterium that causes plague disease in humans and other mammals. Acquisition of infection in humans is by bites of a vector flea harbouring the bacteria or consuming infected rodents usually rats. The prevalence of this disease has not been fully reported hence this research was conducted to determine the prevalence of Yersinia. pestis infection with pathological symptoms locally called "*Okezonwu*" in Abakaliki district, Ebonyi state. A total of sixty-three (63) different clinical samples were collected from patients with different age groups receiving treatment in local herbal centres across the Abakaliki district. A rapid detection test was carried out using Artron f1-*Y. Pestis* is a step dipstick to determine the seroprevalence of the disease. Results showed that 20% of the specimens tested were positive for *Yersinia pestis* infection, with the highest prevalence of 42.9% for age groups between 11-20 years while those in the age categories 1-10 years and 51-60 years were all *Y. pestis* negative. Also, student farmers recorded a 66.67% prevalence, while those who were solely students, civil servants, and minors had no positive sample when considering patients' occupations. Males had the highest prevalence of 22.2% while female patients had a prevalence of 16.67%. This study revealed the presence of Yersinia. pestis in patients suffering from the plague locally known as "*Okezonwu*" in Abakaliki District.

Keywords: Coccobacillus bacterium; occurrence; traditional healing centres.

INTRODUCTION

Yersinia pestis is a gram-negative coccobacillus bacterium that is primarily transmitted through rodents and fleas (Yang *et al*., 2022). It causes the disease plague, a serious and potentially fatal illness characterized by symptoms such as fever, chills, weakness, and swollen lymph nodes (Esmaeili *et al*., 2023). Plague has been responsible for numerous devastating pandemics throughout human history, including the Black Death which killed an estimated 75-200 million people in Europe during the 14th century (WHO, 2022). According to several studies, plague is a disease that is primarily spread by fleas which infest rodents (Mahmoudi *et al*.,

2021). Studies conducted on rodent populations, such as prairie dogs in the United States (Roth, 2019) and great gerbils in Kazakhstan (Reijniers *et al*., 2012), have revealed high seroprevalence rates. This highlights their potential role as sources of human infections. Humans can become infected through contact with infected animals, flea bites or droplet infection (Eisen and Gage, 2009; Banda *et al*., 2022). These infections are incidental, as humans are not the primary hosts of Yersinia pest diseases.
Seroprevalence studies are critical in providing information about the exposure and immune response of populations to Yersinia pestis, which can aid in understanding the epidemiology and potential risk factors of plague outbreaks (Esmaeili *et al*., 2023). These studies involve detecting the presence of specific antibodies against *Y. pestis* in serum samples from individuals or populations (Esmaeili *et al*., 2023). These studies can provide insights into the exposure rates, transmission dynamics, and potential pathogen reservoirs, as noted by Williamson *et al.* (2020). Studies have been conducted in areas where plague is endemic to assess the extent of exposure to *Y. pestis* (Zhou *et al*., 2014). For example, a study in the Ituri region of the Democratic Republic of Congo discovered a seroprevalence rate of 15.3% among the local population, suggesting a significant level of exposure (Bertherat *et al*., 2011). Similarly, a study in the Qinghai-Tibet Plateau region of China reported a seroprevalence rate of 13.2% among human populations, indicating ongoing transmission (Zhou *et al*., 2014).

Despite being considered non-endemic in many regions, investigations into the seroprevalence of plague have revealed surprising facts. Notably, a study in the United States detected antibodies against *Yersinia pestis* in certain individuals, implying possible exposure or cross-reactivity with other pathogens (Born *et al*., 2020). Similarly, a study in the Netherlands reported a seroprevalence rate of 1.8 % among healthy blood donors, which raises queries about potential sources of exposure (Jansen *et al*., 2009). These findings suggest that plague might not be as rare as previously thought, and public health measures should be taken to prevent the spread of the disease. Further research is also needed to determine the extent of the risk and develop appropriate control measures.

The plague, caused by Yersinia pestis, can be found on every continent in the world except Australia. It is particularly endemic in third-world countries such as India, Peru, Madagascar, and China (Glatter and Finkelman, 2021). The distribution of plague is geographically localized to areas where landscape and weather conditions favour a high concentration of rodents and fleas (Hieronimo *et al*., 2014). The World Health Organization has identified natural plague foci in approximately 20 countries across Africa, America, and Asia. WHO reported 40 deaths and 80 other cases on the island of Madagascar as of November 2014, with the first known case of the outbreak reported in late August 2014 (WHO, 2021). Most human cases occur during epizootic periods when highly susceptible hosts die in large numbers, forcing fleas to feed on alternative hosts, including humans (CDC, 2023).

The causative agent of the plague, Yersinia pestis, continues to persist in several African regions, including Nigeria (Ziwa *et al*., 2013; Lotfy, 2015; Banda *et al*., 2022). The investigation carried out by the World Health Organization in collaboration with Madagascar's Ministry of Health in February 2015

showed that the plague season on the Island is from September to April (WHO, 2015). The investigation reported two cases (one probable and one confirmed). In Antananaerio district of Madagascar, altogether 263 cases including 71 deaths have been reported to date, representing a case fatality rate of 27% (WHO, 2015). However, the available data on the prevalence of the disease in Ebonyi state and Nigeria in general is limited and warrants further investigation. This information gap was highlighted by Ogbulu *et al.* (2021). Given the potential severity of the disease and its impact on public health, it is essential to gather more data on the prevalence and distribution of the disease in Ebonyi State to inform effective prevention and control measures (Ogbulu *et al.*, 2021). To address this gap, we conducted this research on the seroprevalence of *Yersinia pestis* among patients attending traditional herbal centres in the Abakaliki district of Ebonyi State.

MATERIALS AND METHODS

Study Area

This study was carried out in herbalist centres located in the Abakaliki district of Ebonyi State. Abakaliki district comprises parts of Ebonyi, Abakaliki, Ezza North, Ohaukwu, and Ezza South Local Government Areas. It is an urban centre with administrative, commercial, and industrial activities. It is located on longitude 80 6' 6' E and Latitude 60 22' 28" N. Abakaliki district is equally located in the lower belt of Nigeria and situated on a high land with tropical rain forest as its vegetation. It occupies the central part of Ebonyi State and is predominantly an agricultural zone. Abakaliki district covers an area of approximately 51 square km. There are several rivers and streams in the area namely Ebonyi River which is the largest and is a tributary of Cross River. Abakaliki district is about 84 km east of Enugu; it is bordered on the west by Enugu State. In the North and South, it shares boundaries with Benue and Abia State respectively (Obasi *et al.*, 2023).

Methods

The authors recruited patients attending local herbal centres in Abakaliki District, between April and September 2016. Before recruiting the patients into the study, the consent of the patient was diligently sought and successfully obtained. This involved providing them with comprehensive information about the study's purpose, procedures, potential risks, and benefits, and ensuring that each patient fully understood and voluntarily agreed to participate. The study area was chosen based on the presence of patients seeking treatment for "*Okezonwu*" a local term referring to plague-like symptoms. Using a stratified multistage sampling method, the research scientist collected a total of 64 samples consisting of blood, urine, stool, sputum and bubo fluid, from 36 patients attending local herbal centres. Blood samples were collected through veni puncture and added into an EDTA bottle for preservation. The sputum, urine and faeces were collected into a sterile universal plain container. Bubo swabs were collected using sterile swab sticks and caped. The samples were taken to the Medical Laboratory Science Department Laboratory of Ebonyi State University, Abakaliki for analysis within three (3) hrs. The samples were analyzed using The Artron One Step *Yersinia pestis* Plague Test Kit (ARTRON). It was used to detect *Yersinia pestis* antigen in the collected samples.

The test was done by immersing a strip from the kit into the sample with buffer following the manufacturer's instruction. The strip was taken out when the sample had migrated to

the test window (10 seconds) and then laid flat on a clean noon absorbent surface with the max side facing up. It was then observed for the appearance of double bands (pink) within the test window. The presence of a double band is indicative of a positive result. The test analysis was performed within three hours of sample collection (Rajerison *et al*, 2009).

Specimen Preparation and Analysis

The blood specimen collected was analyzed within 3 hours of collection. A thin smear was made from the blood and the remaining sample was centrifuged at 1,500 x g for 10 minutes and the plasma was carefully transferred to a sterile tube. The thin smear was made on a sterile, grease-free slide and was allowed to air dry. Then it was fixed using absolute (100%) methanol by dipping the slide into the methanol for five seconds. It was then stained using Giemsa stain for 10 min. After that, it was rinsed with distilled water and allowed to air dry. A drop of immersion oil was applied to it and it was examined using a 100x objective lens (oil immersion objectives). A strip from the Artron One Step *Yersinia pestis* Plague Test Kit (Artron) was used to test for the presence of *Yersinia pestis* antigen in the harvested plasma. It was immersed into plasma as instructed by the manufacturer. The strip was taken out when the sample had migrated to the test window (10 seconds) and then laid flat on a clean non-absorbent surface with the max side facing up. The Presence of a double band (pink) which indicates a positive result was observed within 15 minutes.

***Sputum*:** The specimens collected in a universal container were mixed with Iml of the buffer by vigorous shaking. A strip from the Artron One Step *Yersinia pestis* Plague test kit was immersed into the sputum mixed with sample buffer as instructed by the manufacturer. The strip was taken out when the sample had migrated to the test window (10 seconds) and then laid flat on a clean non-absorbent surface with the max side facing up. The presence of a double band (pink) indicates a positive result was observed within 15 minutes.

***Faeces*:** An Eppendorf stick was used to collect about 0.3 g of the faecal specimen and added to the vial of buffer and mixed up. The mixture was used for the test. A strip from the Artron One Step *Yersinia pestis* Plague Test Kit is immersed into the faeces dissolved with buffer as instructed by the manufacturer. The strip was taken out when the sample had migrated to the test window (10 seconds) and then laid flat on a clean non-absorbent surface with the max side facing up. The presence of a double band (pink) indicates positive results and was also observed within 15 minutes.

***Urine*:** The urine specimens obtained were tested directly with a strip from the Artron One Step *Yersinia pestis* Plague Test Kit, within 2 hours of collection, by immersing it into the urine specimen as instructed by the manufacturer. The strip was taken out when the specimen had migrated to the test window (10 seconds) and then laid flat on a clean non-absorbent surface with the max side facing up. The presence of a double band (pink) indicates a positive result and was observed within 15 minutes.

***Swabs*:** The bubo swabs were placed in 1ml of buffer in a sterile Eppendorf. when the remaining fluid is expressed from the swab by pressing it against the wall of the tube. A strip from the Artron One Step *Yersinia pestis* Plague Test Kit was immersed into prepared

bubo fluid as instructed by the manufacturer. The strip was taken out when the sample had migrated to the test window (about 10 seconds) and then laid flat on a clean non-absorbent surface with the max side facing up. The presence of a double band (pink) which indicates a positive result was observed within 15 minutes.

RESULTS

Out of 36 patients examined in this study, patients belonging to the age group of 11-20 years showed the highest prevalence of 42.86% while those of the age group 1-10 years and 51-60 years had no positive result (Table 1). There was a progressive increase in the prevalence of the infection with advancement in age, starting from 21 to 50 years (Table 1). However, there was no significant difference in infection prevalence concerning age groups (P=1.000) - Table 5. Age group-specific prevalence of the infection indicated an overall prevalence of 19.44 %.

Out of 18 males tested, 4 tested positive for *Y. pestis*, resulting in a prevalence of 22.22% (Table 2). Among 18 females, 3 tested positive, yielding a prevalence of 16.67 %. The overall prevalence for both genders combined was 19.44 % (Table 2).

Among 36 patients, farming was reported as both a primary and secondary occupation by 8 individuals, while 3 were students with farming as a secondary occupation (Table 3). 10 patients were both students, and 5 were solely artisans. The highest prevalence (66.67%) was among students/farmers, followed by sole artisans (40%). No infections were found among sole students, civil/public servants, or minors (Table 3). Statistical analysis showed significant occupation-based seroprevalence (Table 5).

Out of a total of 63 specimens comprising blood (35), urine (21), stool (1), sputum (2) and bubo fluid (4), seven (7) blood samples representing 11.1 % were positive for *Yersinia pestis* infection. All other specimens were negative for *Yersinia pestis* infection (Table 4). The overall prevalence of specimen-specific seroprevalence for *Y. pestis* was 1.59%. Statistical analysis showed that blood had a significant effect (Table 5).

Table 1: Age Group-Based Seroprevalence of *Y. pestis* Infection in Abakaliki District Ebonyi State

Age group (yrs)	Number of people Examined	Number of positive cases	Prevalence (%)
1-10	2	0	0.00
11-20	7	3	42.86
21-30	11	1	9.09
31-40	7	1	14.28
41-50	6	1	16.67
51-60	2	0	0.00
61-70	1	1	100
Total	**36**	**7**	**19.44**

Table 2: Gender-Specific Seroprevalence of *Y. pestis* Infection in Abakaliki District, Ebonyi State

Sex	Number of cases	Number of positive cases	Prevalence (%)
Male	18	4	22.22
Female	18	3	16.67
Total	**36**	**7**	**19.44**

Table 3: Occupational-Based Seroprevalence of *Y. pestis* Infection in Abakaliki District, Ebonyi State

Primary Occupation	Secondary occupation	Number of cases	Number of positive cases	Prevalence (%)
Farmer	Farmer	8	2	25
Student	Farmer	3	2	66.67
Student	Student	10	0	0
Civil servant	Civil servant	8	0	0
Artisan	Artisan	5	2	40
Trader	Farmer	1	0	0
Trader	Trader	1	1	100
Minor	Minor	1	0	0
Total		**36**	**7**	**19.44**

Table 4: Specimen-Specific seroprevalence of *Y. Pestis* infection in Abakaliki District, Ebonyi State.

Specimen	Number Examined	Number Infected	Prevalence (%)
Blood	35	7	20
Urine	21	0	0
Stool	1	0	0
Sputum	2	0	0
Bubo fluid	4	0	0
Total	**63**	**7**	**11.1**

Table 5: Test Statistics of Age, occupation, location, detection and sex of patients attending herbal centres in Abakaliki Districts

	Age of Patients	Occupation of patients	Location of Sampling	Detection of *Y. pestis* F1- Antigen in Serum/Plasma	Sex of Patients
Chi-square	6.000[a]	30.000[c]	1.556[d]	13.444[e]	0.000[e]
df	27	8	3	1	1
Asymp. Sig	1.000	0.000	0.670	0.000	1.000
Monte Carlo Sig	1.000[b]	0.000[b]	0.772[b]	0.000[b]	1.000[b]
Lower Bound	0.920	0.000	0.576	0.000	0.920
Upper Bound	1.000	0.080	0.869	0.080	1.000

a. 28 cells (100.0%) have expected frequencies less than t. The minimum expected cell frequency is 1.3
b. Based on the 36 sampled tables starting with sees 1502173562
c. 9 Cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 4.0
d. 0 Cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 9.0
e. Cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 18.0

DISCUSSION

Plague, caused by the bacterial pathogen Yersinia pestis, has been recognized by doctors and populations as a unique medical condition for centuries. It is the only disease characterized by swollen lymph nodes, called buboes, and has been known to cause deadly epidemics (Barbieri *et al*., 2020). Seroprevalence studies are a valuable tool in *Y. pestis* epidemiological investigation. By detecting antibodies, the study will provide essential insights into how the pathogen spreads, who is most at risk, and how public health interventions can be optimized to prevent future outbreaks (Williamson *et al*., 2020). The seroprevalence of *Yersinia pestis* infection among patients with plague, locally known as Okeezonwu', in Abakaliki District was found to have a lower rate compared to the 27 % recorded in Madagascar by WHO in 2015 (WHO, 2015). This could be linked to low historical exposure to plague outbreaks in Abakaliki and the development of high levels of immunity to *Y. pestis* due to previous exposure in the region. Additionally, there might be genetic differences in the populations of Abakaliki and Madagascar that affect susceptibility to *Y. pestis* infection. Certain genetic traits could confer resistance to the disease, resulting in lower seroprevalence rates.

The observed seroprevalence level demonstrates active *Y. pestis* transmission in Aba-

kaliki District. The prevalence aligns with other African studies that found 7-17% seropositivity (Biey *et al*, 2014; Banda *et al*, 2022). Increasing seropositivity with age reflects higher cumulative exposures. Positive associations between seropositivity and animal contact including swallowing dead rats highlight zoonotic transmission routes for *Y. pestis* (WHO 2022). Poor knowledge indicates a need for community education on plague prevention (Ogbulu *et al.* 2021).

Regarding demographic parameters, the study revealed a significant relationship between *Yersinia pestis* infection and occupation. The highest prevalence was observed among students who also worked as farmers, likely due to their exposure to flea bites during farming activities (Zhou *et al.*, 2006; Ke *et al.*, 2013). Artisans, who predominantly reside in rural areas and may engage in farming or hunting, also exhibited a high prevalence rate. In contrast, individuals solely engaged in civil service or studying showed no incidence of infection, possibly due to their sanitized living environments that are less conducive to hosting plague-susceptible rodents or fleas (CDC, 2014). People who were purely civil servants and students recorded no incidence of the infection. This could be because these classes of people may be living in a highly sanitized environment which does not favour the inhabitation of the deadly rodents or their fleas.

Although not statistically significant, a higher prevalence of *Yersinia pestis* was observed among males compared to females. This finding contradicts that reported by Minnaganti (2016). The elevated prevalence among males could be attributed to their increased exposure to farming activities, which in turn heightens their risk of flea bites compared to their female counterparts. The study has indicated a higher prevalence of *Yersinia pestis* infection among individuals aged 11-20 years, consistent with findings reported by Minnagati (2016) in Brazil. This age group is highly active and likely to engage in farming and hunting activities, thereby increasing their proximity to vectors.

The seroprevalence study in Abakaliki District highlights active transmission of Yersinia pestis, reflecting significant exposure within the community. This rate, though lower than Madagascar's 27% in 2015, aligns with other African regions, suggesting regional variations in exposure and immunity levels. The study underscores the importance of seroprevalence in understanding the epidemiology of plague, revealing critical associations between infection rates and demographic factors such as occupation, age, and gender. The higher prevalence among individuals engaged in farming and artisanal work, particularly among younger males, emphasizes the need for targeted public health interventions, including community education on plague prevention, improved sanitation, and specific protective measures for high-risk groups (Ogbulu *et al*, 2021). These findings are crucial for developing effective strategies to mitigate the impact of *Y. pestis* and prevent future outbreaks. However, it points to the importance of adopting more comprehensive research using a One Health approach to be able to generate results that can be used in policy making and interventions.

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