

SEROPREVALENCE OF *BACILLUS ANTHRACIS* IN JOS AND ENVIRONS

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ABSTRACT

The fears of a possible out break of infection following media reports of intentional release of anthrax spores has drawn attention to the need to establish a baseline information on the prevalence of *Bacillus anthracis* in the cattle, sheep and goat population in Jos. We conducted a survey to determine the prevalence of antibodies to *Bacillus anthracis* in cattle, sheep and goats slaughtered at the Jos Abattoir using the agar diffusion method. The survey involved two hundred animal subjects (160 cattle, 20 each of sheep and goats). Out of the total number of samples screened, 21(10.5%) were seropositive, with antibody titre levels of $\leq 1/80$. Of the 21 seropositive cases, 18 (9.0%) were cattle, 3(1.5%) were sheep and there was none for goats. These findings allay our fears of the possibility of an outbreak of anthrax infection following the consumption of meat from animals slaughtered at the Jos Abattoir.

INTRODUCTION:

Anthrax is an acute infectious zoonotic disease caused by spore-forming bacterium *Bacillus anthracis* and affects wild and domestic animals (cattle, sheep, goats, camels, antelopes and other herbivores). It affects humans when they are exposed to infected animals (1). Historically, human anthrax in its various forms has been a disease of those with close contact to animals or animal products contaminated with *Bacillus anthracis* spores, (2).

Anthrax is a disease of well-documented antiquity. For example, the accounts of the fifth and sixth plagues of Egypt given in the ninth Chapter of Exodus are seen by many as describing instances of systemic and cutaneous anthrax (3).

In humans, anthrax most commonly occurs as cutaneous lesions or boils which usually progresses to a black eschar despite antibiotic

treatment. Pulmonary anthrax (inhalation) and a third form gastrointestinal anthrax usually resulting from ingestion of meat or spore-contaminated food may be observed (4).

Anthrax in animals may occur in the following forms: peracute, acute, subacute and chronic. The peracute form generally found in cattles, sheep and goats at the beginning of an outbreak is characterised by a sudden onset and rapidly fatal course. Some are found dead without any previous signs of the disease (5). In the acute forms, animals exhibit signs up to two days before death (3). The first noticeable sign/symptom is a rise in temperature and both the acute and subacute conditions are characterised by excitement, simulating rabies followed by depression, respiratory distress, trembling, staggering, convulsion and death.

Anthrax has become a current issue because it is considered to be a potential agent for use in biological warfare. The identification of inhalational anthrax in a journalist in Florida on October 4, 2001, marked the beginning of the first confirmed outbreak associated with intentional release of anthrax in the United States (2).

In Nigeria, a member of Senate was reported to have received a suspected mail, which turned out to be a hoax after thorough laboratory investigation at the Bacteriology Laboratory at the National Veterinary Research Institute, Vom. There is therefore a need to establish the prevalence of this potential agent of biological warfare in our environment.

The aim of this study is to investigate the seroprevalence of *B. anthracis* responsible for infection in cattle, sheep and goats in Jos and its environs.

MATERIALS AND METHODS

Sample Collection

Two hundred (200) blood samples from slaughtered cattle, sheep and goats were collected from the Jos Abattoir in McCartney bottles. One hundred and sixty (160) of the blood samples were obtained from cattle while twenty of the blood samples each were collected from sheep and goats. The blood samples were allowed to clot after collection and sera from the clotted blood samples were separated into clean dry Bijou bottles using clean pipettes. The sera were kept at -20°C until they were ready for use.

Experimental Animals

The selected in-bred female Hartley guinea pigs, 350-400g were quarantined for one week. They were bled by cardiac puncture and screened to be sure they had no antibody to anthrax. The clean animals were then kept for the experiment.

Animal Inoculation

The harvested live attenuated anthrax spore suspension in saline was washed using normal saline. Viable count of the spore was determined using Miles & Misra (6) method. 0.2% formalin was added to the washed suspension ensuring that the final concentration of the spore suspension remained approximately 2.0×10^9 cfu/ml. Each guinea pig was given four daily inoculation intravenously of 1cm^3 of the suspension. After two weeks, post last inoculation, the guinea pigs were bled, serum separated and tested for antibody titre. The standard antisera was then stored in deep-freezer until when required as positive control antisera.

Standard Antigen

A large batch of attenuated Sterne strain culture filtrate which had a titre of 1×10^2 cfu/ml in the agar diffusion assay with the standard antiserum was used as a standard control for comparing line patterns of other antigen preparation.

Preparation of Medium for Agar Diffusion Plates.

One gram of agarose containing 0.015m phosphate buffer (pH 7.3), 0.01% thiomersalate and 0.09% NaCl was dissolved in 1000mls of distilled water and was then autoclaved at 121°C for 15 minutes. It was allowed to cool to 50°C and 15ml of the agar medium were dispensed into 90mm Petri dishes. Circular wells were made in the solidified agar with cork borers. The outer wells were 7mm in diameter and 5mm apart and 6mm from the inner well of 5mm in diameter. The plates were stored at 2°C and used as required.

Agar Diffusion Method

An agar diffusion method for titrating antibody against antigen as described by Thorne and Belton (7) was used.

The outer wells were filled with appropriate dilutions of test antisera in saline (1/20,

1/40, 1/80, 1/160) and the plates were held at 2°C for 18-24 hours. The inner well was then filled with a neat solution of antigen after which the plates were kept at room temperature. The plates were observed after 18-24 hours for lines of precipitation by holding over a light against a dark background. The final readings were taken on the second day after titration.

Antibody titre values of 1/80 and below were considered as negative.

Method of Antisera Dilution

One millilitre (1ml) of the working serum was pipetted into the first test tube in the rack containing 9ml of 0.9% saline solution to give a dilution of 1:10. Double dilution process was carried out for the rest of the tubes (i.e. 1/20, 1/40, 1/80, 1/160, 1/320). One millilitre (1) ml from the last test tube was then discarded into a disinfectant jar. Equal amounts of the respective dilutions were then used to fill the outer wells on the agar medium as was done above.

RESULTS

Two hundred serum samples were titrated against filtrate of Sterne strain of *B. anthracis* in nutrient broth. The highest dilution of test antiserum that prevented formation of a visible precipitin line was taken as the end point. The results obtained showed that a total of 21(10.5%) serum samples were positive. Of the total number of positive cases 18(9.0%) were from cattle (Table 1) while 3(1.5%) were from sheep (Table 2). There were no positive cases from goats (Table 3).

Six (3.75%) of 160 cattle serum samples tested had titres of 1:80, 8(5.0%) had a titre of 1:40, 4(2.5%) had a titre of 1:20 while none had titre >1:160. Only 1(5%) out of the 20 serum samples tested for sheep had titre of 1:80 and 2(10%) had titre values of 1:20. All the goats had no detectable antibody to *B. anthracis*. Titre values of 1:80 and

below have been used as standards for evaluation that animals have been vaccinated while titre values of 1:160 and above are suggestive of infection.

Table 1: Antibody levels to *Bacillus anthracis* in Cattle.

N=160

Sera Sample Size	1/20	1/40	1/80	1/160	1/320	1/640
4(2.5%)	+ve	-ve	-ve	-ve	-ve	-ve
8(5.0%)	+ve	+ve	-ve	-ve	-ve	-ve
6(3.75)	+ve	+ve	+ve	-ve	-ve	-ve
142(88.75)	-ve	-ve	-ve	-ve	-ve	-ve

Table 2: Antibody levels to *Bacillus anthracis* in Sheep.

N=20

Sera Sample Size	1/20	1/40	1/80	1/160	1/320	1/640
2(10.0%)	+ve	-ve	-ve	-ve	-ve	-ve
1(5.0%)	+ve	+ve	+ve	-ve	-ve	-ve
17(85.0%)	-ve	-ve	-ve	-ve	-ve	-ve

Table 3: Antibody levels to *Bacillus anthracis* in Goats.

N=20

Sera Sample Size	1/20	1/40	1/80	1/160	1/320	1/640
20(100.0%)	-ve	-ve	-ve	-ve	-ve	-ve

DISCUSSION

Results obtained from this study showed that 10.5% of the study population (animals) were seropositive for antibodies to *B. anthracis* with antibody levels 1:80. The low titre values suggest that animals have been vaccinated against the infection. The percentage positive was higher in cattle (9.0%) than in sheep (1.5%). The local herdsmen could attribute this to the routine

vaccination of cattle with the Anthrax spore vaccine.

In a study in Zambia, 365 specimens of various tissues from animals and surface water over a 5 year period (1987-1991) were examined for anthrax, 85 animals were positive. Of this number, 35 were in domestic animals comprising of 33 cattle, 1 sheep and 1 pig (8). Over 100 human deaths from anthrax, usually associated with eating infected meat were recorded in the Western and North Western provinces of Zambia between 1990 and 1993.

Sheep are usually not vaccinated against Anthrax in our environment but the few positives may be due to grazing on contaminated feed/grasses resulting from spills from the vaccine during vaccination of cattle. This is possible since the sheep are reared alongside the cattle. The fact that no goats, which are reared separately, recorded a zero percent seropositivity gives credence to the earlier assertion.

It has been mentioned earlier that the disease can affect humans when they are exposed to infected animals or tissues from these infected animals. This study suggest that the animal population examined (cattle, sheep, and goats) may not be capable of causing anthrax infection in Jos and its environs when ingested. There also has been no record of any outbreaks among butcher in the Jos Abattoir.

Our findings in this study suggest that due to an effective vaccination scheme against anthrax in cattle in Jos, Nigeria, there has been no reported of anthrax infection. We therefore allay fears of a possible outbreak of anthrax in the human population from animal sources.

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