ABSTRACT

Bird mortality has been frequently reported among poultry farmers, and the prevailing circumstances in the poultry farms seem most favourable for incidental cross-infections, notably Chlamydia infections among poultry farmers. This study therefore has investigated the various circumstances in the poultry farms are likely to result in poor economic gains. Better hygienic measures, like maintenance of a healthy environment inside and outside the poultry shed, protection of birds from non-infectious agents such as parasitic or fungal infections, and proper disposal or sanitation of dead birds. However, Chlamydia trachomatis persists longer and more often asymptomatic than Neisseria gonorrhoeae in the urogenital tract of men and women. About 20% of all chlamydia infected women suffer from partial or complete tubal occlusion. Chlamydia trachomatis is the leading cause of female infertility, but most of these women never experienced any clinical sign of pelvic inflammatory disease. About 20% of all chlamydia infected women suffer from partial or complete tubal occlusion. Chlamydia trachomatis is the leading cause of female infertility, but most of these women never experienced any clinical sign of pelvic inflammatory disease. About 20% of all chlamydia infected women suffer from partial or complete tubal occlusion. Chlamydia trachomatis is the leading cause of female infertility, but most of these women never experienced any clinical sign of pelvic inflammatory disease.

Keywords

Mortality, Pathogenic, Microorganisms, Poultry

INTRODUCTION

Chlamydiae are obligate intracellular bacterial pathogens that cause pneumonia, sexually transmitted infection, trachoma, and psittacosis (Brizolara et al., 1990; Anyanwu and Nnadozie, 1993; Mishori et al., 2012; Idu et al., 1998). With the constant increase in poultry meat consumption worldwide and the large variety of poultry meat products and consumer demand, ensuring the microbial safety of poultry carcasses and cuts is essential. Bacteria from the air and the environment can contaminate broiler meat (Buchanan et al., 2015). The skin of poultry carcasses and cuts is directly in contact with air and equipment surfaces and is therefore easily contaminated. In fresh meat, bacteria are present on the surface rather than in the meat. However, in processed products such as those which have been marinated, bacteria can migrate into the muscles (Rouger et al., 2017). Disease out-break, increased mortality and higher percentages of cull birds could adversely affect profitability of egg type layers. Diseases of chicken are mostly infectious in nature and therefore, wide variability in losses due to such diseases is expected in egg type layers. Clinically genital chlamydial infections resemble gonorrhea (dysuria, discharge, irregular bleeding, dyspareunia, perihepatitis) and may be mistaken for appendicitis. However, Chlamydia trachomatis persists longer and more often asymptomatic than Neisseria gonorrhoeae in the urogenital tract of men and women. About 20% of all chlamydia infected women suffer from partial or complete tubal occlusion. Chlamydia trachomatis is the leading cause of female infertility, but most of these women never experienced any clinical sign of pelvic inflammatory disease (Mishori et al., 2012; Resnikoff et al., 2004; Mak et al., 2005). E. coli and other bacterial infections are more prevalent in between the age of 6-11 weeks than at later stages of life (18-22 weeks). Birds exposed to climatic extremes and seasonal conditions are characteristically more prone to all types of diseases than others. Such losses could be attributed to adverse climatic conditions to which the chickens were exposed, lowering the immune response against diseases. In fact, these losses are heavier and would contribute irreparable losses to flock owners if not properly handled, as increased level of mortality will result in poor economic gains. Better hygienic measures, like maintenance of a healthy environment inside and outside the poultry shed, protection of birds from extreme climatic conditions, restriction of visitors and wild animals, proper cleaning and disinfection of houses, equipment and workers, and appropriate floor and house.
construction will help in reducing losses due to mortality. In addition, assurance of healthy drinking water, effective vaccination against disease, antibiotic therapy, chlorine treatment and filtration of water tanks would reduce mortality many folds (Mukherjee and Khampurkar, 1994). As for the genus Chlamydia, in addition to the old ones (i.e., *C. trachomatis*, *C. suis*, *C. muridarum*, *C. pneumoniae*, *C. abortus*, *C. caviae*, *C. felis*, *C. psittaci*, and *C. pecorum*), five new species, characterized through whole-genome sequencing (WGS), have been introduced in the last decade. Specifically, *C. avium*, *C. gallinacea*, and *C. buteonis* were identified in birds while *C. serpentis* and *C. poikilothermis* in snakes (Chaiwattanarungruengpaisan et al., 2021). In a study, two out of the three *C. psittaci* rural farms were also positive for *C. gallinacea*. Mixed infection with different chlamydial species is well known and documented (Hejne et al., 2021). *C. avium* was not detected in any poultry farm in study. In Europe, this chlamydial species has been primarily described in wild pigeons and psittacines (Popelin-Wedlarski et al., 2021). Additionally, new candidate species and taxa have been lately described in fishes and reptiles. More insights on *C. gallinacea* pathogenicity have been recently reported by experimental infections in chickens, showing that infection with the NL_G47 strain does not lead to acute clinical disease after oral inoculation, and the bacteria mainly reside in the epithelium of the gut (Popelin-Wedlarski et al., 2021). *C. trachomatis* is the leading cause of preventable blindness because cases of trachoma have been successfully treated with antibiotics (Sharma et al., 2002; Messinger et al., 2015).

Clinically genital chlamydial infections resemble gonorrhoea (dysuria, discharge, irregular bleeding, dyspareunia, perihepatitis) and may be mistaken for appendicitis. However, *Chlamydia trachomatis* persists longer and more often asymptomatic than Neisseria gonorrhoeae in the urogenital tract of men and women. About 20% of all chlamydia infected women suffer from partial or complete tubal occlusion. *Chlamydia trachomatis* is the leading cause of female infertility, but most of these women never experienced any clinical sign of pelvic inflammatory disease (Mishori et al., 2012; Resnikoff et al., 2004; Mak et al., 2005).

Objective of the research include:

(a) To determine the mortality rate of the birds in the poultry farms that has been associated with Chlamydia infection and other infections.

(b) To determine the distribution of Chlamydia infection and other infections among the poultry farmers in Abia State Nigeria,

(c) To determine the demographic characteristics (gender and age) individual lifestyles and residential exposure

(d) To identify the extent to which the infection could results to trachoma and other genital diseases.

(e) To determine the percentage yield loss of the poultry products as a result of the associated infections on the birds and workers in the farms under investigation.

**Materials and Methods**

**Area of Study**

The study areas include Aba and Umuahia; which are located in South East of Nigeria.

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![Figure 1: Map of Abia State showing the study area](https://journals.e-palli.com/home/index.php/ajaset)
Collection of Samples
A total of 127 poultry farmers of whom 52% were females while 48% were males, age between 21 and 60 years participated in this investigation. For the control, equal number university students (35 females and 35 males), and aged 19-42 years were also studied. This study population was chosen by random sampling technique. Each person was examined for signs of trachoma using the World Health Organization (WHO) simplified trachoma grading as the criteria for evaluation.

Specifically designed questionnaires, and interviews were also dispensed to the participants to identify socio-economic and behavioral practices, beliefs and artifacts that poultry farmers, communities and student participants have adopted to reduce the incidence of Chlamydial infections.

Cervical specimens were collected by scraping the mucosa using readily available but less traumatic swabs. Also nose and mouth secretions from sick birds and dead birds were collected using swab stick.

Microbiological culture and Serological test/antigen test for direct detection of Chlamydia immunofluorescence were then carried out on the samples using the Chlamydia kit.

Isolation microorganisms
Standard Microbiological techniques were employed in the enumeration of the probable microorganisms present in the farms. Samples were processed according to (Onyeagba, 2015). Already Swabbed samples were added into 9ml of sterile physiological saline, serial dilution carried out to 10-5 dilution. One ml of the solution was inoculated onto already prepared and solidified Nutrient, MacConkey agar, Sabouraud Dextrose agar (SDA), Salmonella-Shigella agar (SSA) using spread plate method, and incubated for 24hours at 370C, SDA plates were incubated at 250C for 3-7 days (Onycagaba and Iroha, 2006).

Identification of bacterial and fungal isolates
Bacterial isolates were identified base on their Gram staining and biochemical characteristics (indole test, sugar fermentation, methyl red test, voges- proskauer test, catalase test, coagulase test, citrate test) and compared with criteria in Bergey’s Manual of Determinative Bacteriology by Buchanan and Gibbons (1974). Fungal isolates were identified base on their morphological characteristics on SDA and Lactophenol cotton blue stain identification and compared with criteria in Barnett and Hunter (1972).

RESULTS
The research conducted to investigate the bird mortality and incidence of chlamydial infections among poultry farmers in Abia State, Nigeria revealed some results which were shown below. A total of 127 poultry workers were included in the study: 49 % from Aba area and 51 % from Umuahia area (Table 1). Most poultry workers/ farmers were women (52%) and middle age of 21-60.

The demographic characteristics were analysed and shown in Table 1. For Umuahia and Aba area, the male in age group of 41 – 50 had the highest number of poultry workers than other groups while the least was observed in ages 21-30 for Aba area.

Organisms isolated from the farm environment include Salmonella sp, Escherichia coli, Staphylococcus aureus, Streptococcus sp, Bacillus sp, Aspergillus sp and Penicillus sp (Table 2). The result obtained for the incidence of chlamydial infection among poultry farmers were presented in Tables 2 and 3. For Umuahia zone, the male had a percentage positive reaction of 30.77% while the female had 14.70%. For Umuahia zone, 30.43% and 24.32% positive reaction to the antigen test were recorded for male and female respectively (Table 3 & 4).

Keys: HVS: High vaginal Swab ;ECS: End Cervical Swab

Table 1: Demographic characteristics (gender, age) of the Poultry Workers

| Ages     | Aba Male | Aba Female | Umuahia Male | Umuahia Female | Total |
|----------|----------|------------|--------------|----------------|-------|
| 21 -30   | 0        | 2          | 3            | 2              | 7     |
| 31 - 40  | 07       | 13         | 3            | 11             | 34    |
| 41 - 50  | 14       | 10         | 16           | 12             | 52    |
| 51 – 60  | 08       | 08         | 10           | 08             | 34    |
| TOTAL    | 29       | 33         | 32           | 33             | 127   |

Table 2: Organisms isolated from the various sampling area (Aba & Umuahia)

| Organisms   | Air | Urine | Nose Swab |
|-------------|-----|-------|-----------|
| Salmonella sp | +   | -     | +         |
| Escherichia coli | +   | -     | +         |
| Staphylococcus aureus | +   | +     | +         |
| Streptococcus sp | +   | +     | +         |
| Bacillus sp | +   | +     | +         |
| Chlamydia sp | -   | -     | +         |
| Aspergillus sp | +   | -     | +         |
| Penicillus sp | +   | -     | -         |
| Rhizopus sp | +   | -     | -         |
| Mucor sp | +   | -     | -         |

Table 3: Incidence of Chlamydial infection among poultry farmers in Aba zone

| Sex | Samples | Sensitivity |
|-----|---------|-------------|
|     | Urine   | HVS | ECS | R  | NR | R%  | NR% |
| Male | 26      | 0   | 0   | 8  | 18 | 30.77 | 69.23 |
| Female | 12     | 20  | 2   | 5  | 29 | 14.71 | 85.29 |
| Total | 38      | 20  | 2   | 13 | 47 | 21.67 | 78.33 |

Table 3: Incidence of Chlamydial infection among poultry farmers in Umuahia zone

| Sex | Samples | Sensitivity |
|-----|---------|-------------|
|     | Urine   | HVS | ECS | R  | NR | R%  | NR% |
| Male | 23      | 0   | 0   | 7  | 16 | 30.43 | 69.57 |
| Female | 24    | 13  | 0   | 9  | 28 | 24.32 | 75.68 |
Swab; R: Reactive; NR: Not Reactive

The bird mortality rate for the various farms studied revealed percentage of 13 to 22 for Aba zone samples while Umuahia samples ranged from 12.4% to 19%. Highest mortality rate was observed for those with smaller number of birds (Table 5).

Frequency of disease symptoms showed that the poultry farmers or workers had varying symptoms associated with their occupation. The female workers were more susceptible to most symptoms than the male workers (Table 6). The complications conjunctival and deep scar tissue (49%),

Table 5: Bird Mortality Rate in Poultry farms at Aba and Umuahia Area

| Poultry size     | Aba         | Umuahia      |
|------------------|-------------|--------------|
|                  | Average Deaths | % Deaths | Average Deaths | % Deaths |
| 100              | 22          | 22          | 19          | 19          |
| 200              | 27          | 13.5        | 35          | 17.5        |
| 300              | 43          | 14.33       | 47          | 15.67       |
| 400              | 54          | 13.5        | 53          | 13.3        |
| 500 and Above    | 65          | 13          | 62          | 12.4        |

Table 6: Frequency of Disease Symptoms shown by poultry farmers

| Parameters                        | Ages 20 - 30 | Ages 31 - 40 | Ages 41 - 50 | Ages 51 - 60 | % |
|-----------------------------------|--------------|--------------|--------------|--------------|---|
|                                   | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Total |
| Lid shortening                    | -    | -      | 7    | 13     | 9    | 22     | 8    | 10      | 54.33 |
| Acute respiratory infection       | -    | -      | 9    | 15     | 9    | 23     | 7    | 12      | 59.05 |
| Sexually transmitted disease      | -    | -      | 5    | 9      | 7    | 11     | 8    | 11      | 40.15 |
| Conjunctivitis                    | -    | -      | 7    | 15     | 8    | 17     | 6    | 10      | 49.60 |
| Pneumonia                         | -    | -      | 3    | 8      | 5    | 11     | 3    | 7       | 29.13 |
| Trichiasis                        | -    | -      | 7    | 5      | 18   | 5      | 5    | 4       | 34.64 |
| Diarrhea                          | -    | -      | 7    | 11     | 8    | 12     | 7    | 9       | 42.51 |
| Shortness of breath               | -    | -      | 7    | 9      | 7    | 10     | 5    | 8       | 36.22 |
| Headache                          | 0    | 2      | 8    | 10     | 13   | 14     | 12   | 13      | 56.69 |
| Thrembling of hand                | -    | -      | 7    | 10     | 12   | 13     | 12   | 12      | 51.96 |
| Feeling of general exhaustion/ Nausea| -    | -      | -    | -      | 10   | 13     | 13   | 10      | 36.22 |
| Weakness of Arm                   | -    | -      | -    | -      | 11   | 12     | 11   | 12      | 36.22 |

which probably led to cicatricial entropion, trichiasis (35%), lid shortening (54%) in poultry farmers. Chronic conjunctivitis, sexually transmitted disease, conjunctivitis, diarrheae, shortness of breath and weakness of arm were present in most of the poultry farmers compared to the control.

DISCUSSION

Seven dominant bacteria and three fungal isolates were isolated. *Escherichia coli, Staphylococcus aureus, Klebsiella sp, Pseudomonas sp, Streptococcus sp, Salmonella sp, Bacillus sp, Aspergillus niger, Aspergillus flavus, Penicillium sp, Rhizopus sp.* The same organisms were isolated from the two areas. All the isolates were found in the air inside the poultry farms. The urine samples only had three organisms namely, *Escherichia coli, Staphylococcus aureus and Streptococcus species.* From the swabs, all the organisms were isolated except for *Pseudomonas species* and *Rhizopus species.* The result obtained showed that the two study areas were highly polluted, though the frequency of isolation of the organisms varied.

Most of the microorganisms isolated in this study have been associated with diseases of the poultry farm. *Salmonellosis* is caused by bacterium of the genera *Salmonella,* this infection is common in two weeks old chicks and ducklings, *Salmonella* gastroenteritis of human have been associated with consumption of infected birds, hence the infection of birds with *Salmonella* has been attributed to contaminated feeds. Because salmonellae are a public health hazard, it is essential that efforts be directed toward their elimination from eviscerated poultry. Poultry is still considered to be the single most important source of salmonellae. Chickens and turkeys accounted for about 28% of the incidences of Salmonella in domestic animals and their environment, but only about 19% of the incidence of human food borne isolations...
Isolation of E. coli a coliform is an indication of fecal material contamination which can be associated with poor hygiene (Osara et al., 2017; Okogun et al., 2016). Bacillus sp and Staphylococcus aureus that were implicated in the study could be responsible for microbial disease outbreak in the poultry farm. The isolation of fungi genera (Aspergillus, Mucor, Rhizopus and Penicillium) which are mostly mycotoxigenic could be linked to the cereal raw materials used in feed formulation. Mycotoxins are economically important toxins which are immunosuppressive and can result to low poultry production. Aspergillus sp can also cause aspergillosis in birds, leading to mycotoxin production in poultry feeds (Osara et al., 2017). The principal fungus causing Aspergillosis in poultry is Aspergillus fumigatus. Transmission is by inhalation of fungus spores from contaminated litter (e.g. wood shavings, straw) or contaminated feed. Rhizopus spp and Mucor sp were the predominant fungi in this study which might cause deterioration of the feeds ingredients making less nutrients available for the birds. Also some species of Rhizopus are mycotoxigenic. The high level of fungi obtained in this study can be as a result of low water activity of the poultry feed (Osara et al., 2017). Generally most of the microorganisms isolated in this study were pathogenic microorganisms. The chlamydial antigen reaction had higher percentage in male than in women as compared to the number of poultry workers analysed. Also the samples obtained in Aba revealed higher percentage than the Umuahia samples. This could be as a result of the population density in Aba Area. Chlamydia pneumoniae is not as fully understood as other species of the genus of chlamydiae which cause sexually transmitted disease (C. trachomatis) or conjunctivitis (C. psittaci), but it is far more widespread and may be far more dangerous. A high proportion of adults from different countries are positive for antibodies to C. pneumoniae, implying a high prevalence of these infections. Several lines of evidence suggest that Chlamydia pneumoniae can make its way into the walls of various blood vessels, linger for years inducing the inflammation and immune reaction that causes heart attacks and strokes. As it is shown by its designation, Chlamydia pneumoniae has been established as an important human respiratory pathogen causing both endemic and epidemic disease, including pneumonia, bronchitis, pharyngitis and sinusitis. Humans are susceptible, especially older and immunosuppressed individuals who are at a higher risk. Chlamydiosis in humans is an occupational disease of turkey growers, haulers, and processing workers in the live-bird areas and of workers in pet-bird aviaries although the incidence is rare (Butcher et al., 2015) Genital chlamydial infection is the commonest curable sexually transmitted disease in England and Wales. It is a major cause of pelvic inflammatory disease, ectopic pregnancy, and infertility (Macmillan and Templeton, 1999; Fenton, 2000; Mak et al., 2005). Chlamydia trachomatis infection is the most common sexually transmitted infection. It can cause pelvic inflammatory disease and subsequently result in tubal infertility. Chlamydia trachomatis infection in pregnancy can also cause neonatal conjunctivitis and pneumonia (Chotnopparatpattara et al., 2003; Bakken et al., 2004; Mishori et al., 2012). When activated by stress factors, chlamydial infection may take a severe, systemic and sometimes fatal course of disease. Classification of chlamydiae had traditionally been based on host and/or disease association. In a new proposal for classification, similarities of the 16S and 23S ribosomal RNA genes are the basis of a new taxonomy for the order Chlamydiales (Everett et al., 1999).

More men in Aba volunteered their urine samples than females. A total of 38 urine samples, 20 HVS and 2 ECS samples were obtained. A total of 47 urine and 13 HVS samples were collected in Umuahia. There were no ECS samples. The highest mortality rate of 19.0 percent was observed in the farms with about 100 birds, while it was least (12.4 percent) in poultries with more than 500 birds. This is possibly due to the fact that, farmers with large numbers of birds tend to be more focused on the farms knowing that the outbreak of any disease could wipe out entire their means of livelihood. The poultries with smaller numbers of birds usually are more of hobbies to the owners who may not undertake all necessary investment needed to take care for the birds. The disease symptoms were exhibited across the entire poultry workers age groups. The poultry workers ages ranged from 21 years to 60 years. The most affected group were the females within the age group of 41-50 years. The least affected was the 21-30 years group. Women in their middle age are more vulnerable to infections as a result of their role in reproduction (stress and strain of child birth), domestic chores and their physiological make-up etc (Maher, 2013).

Bird mortality rate was higher in poultry farm with smaller number of birds and this could be attributed to lack of proper education on poultry management. Better hygienic measures, like maintenance of a healthy environment inside and outside the poultry shed, protection of birds from extreme climatic conditions, restriction of visitors and wild animals, proper cleaning and disinfection of houses, equipment and workers, and appropriate floor and house construction will help in reducing losses due to mortality (Khurshid et al., 1995).

Mortality rate may rise due to disease, predation or high temperature. The mortality rate of small chicks (up to eight weeks of age) is about 4 percent; that of growers (between eight and 20 weeks of age) is about 15 percent; and that of layers (between eight and 20 weeks of age) is about 12 percent. The average mortality rate of a flock is from 20 to 25 percent per year. Selective culling is usually used by the farmers in removing individual unproductive or sick birds. Chlamydia was also detected from some of the culled and dead birds indicating that Chlamydia could be responsible for the death of birds in such
farms. The clinical symptoms observed which include lid shortening, conjunctivitis, trichiasis are indicative of trachoma, chlamydia - sexually transmitted disease and pneumonia etc.

CONCLUSION
The study revealed the presence of some pathogenic organisms which could be responsible for disease outbreak in the poultry farms resulting in bird mortality. The presence of these microorganisms is a major health concern to the poultry workers & birds. Poultry farmers are also susceptible to chlamydial infections and the effects could be prevented if proper sanitary and prophylactic measures are taken. It was concluded that the best way forward in improving the control of the infection of trachoma and its associated complications at community levels is to establish effective local eradication programs, adopt preventive poultry farming process and general hygiene in handling poultry products, strengthen the surveillance system for monitoring changes in the prevalence of the disease; develop specialized expertise in recognizing infections and thus early prevention.

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