

***Escherichia coli* Infections in Chicken Broilers in Sebele, Gaborone, Botswana**

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Abstract: *Escherichia coli* was isolated from broiler chickens submitted to the Botswana College of Agriculture Animal Health Clinic from January 2001 to December 2002 either as live, sick or dead birds. The bacterium was recovered from 603 (66.6%) of the 906 chickens subjected to a post-mortem examination. Infection due to *E. coli* accounted for 50% of all broiler mortality. The number of cases increased almost threefold over a 12 month period. The rate of infection was significantly higher ($p = 0.05$) during the rainy season ($74.1 \pm 6.9\%$) than during the dry season ($57.0 \pm 8.7\%$). Antibiotic sensitivity showed that more than 50% of all the *E. coli* isolates were resistant to 5 out of 8 the antimicrobial agents tested. The magnitude of resistance was highest for ampicillin, cephalothin, sulphatrid and tetracycline. The *E. coli* isolates were sensitive to cotrimoxazole (sulphamethoxazole plus trimethoprim) and colistin sulphate. Results from this study, showed a high prevalence rate of *E. coli* isolates with variable resistance to a wide range of antimicrobial agents. The dynamics of the infection, possible predisposing factors, antimicrobial sensitivity test on isolates and the public health implications as well as remedial action to circumvent antibiotic resistance are discussed. This is the first report of *E. coli* infection among chickens uncomplicated by concurrent infection due to other pathogens.

Key words: *Escherichia coli* colisepticaemia, antimicrobial resistance, cotrimoxazole and colistin sulphate, drugs of choice

INTRODUCTION

Breeding chickens for rapid growth has resulted in reduced immunocompetence with subsequent reduction in resistance to disease (Knap and Bishop, 2000; Altekruse *et al.*, 2002). The growth of the poultry production industry is hampered by several factors including diseases such as colibacillosis which is a known significant disease of poultry in the developing countries (Jordan, 1994). Chickens under intensive production are susceptible to colibacillosis caused by a bacterium, *Escherichia coli*. The bacterium has been cited as an important cause of morbidity and mortality in poultry, causing significant economic losses to the poultry industry (Allan *et al.*, 1993). According to these authors, certain pathogenic strains of *E. coli* may cause colibacillosis via the respiratory tract of chickens.

The systemic form of this infection referred to as colisepticaemia occurs when large numbers of pathogenic *E. coli* gain access to the blood stream from the respiratory tract or intestine (Blanco *et al.*, 1998). This condition may present as ruffled feathers and dyspnoea (Saif, 2003), pericarditis, air sacculitis, egg peritonitis; omphalitis and salpingitis (Barnes and Gross, 1997). The organism is ubiquitous and large numbers of *E. coli*

are maintained in the poultry house through faecal contamination of water and feed (Jordan, 1994).

Factors thought to predispose chickens to *E. coli* infections include presence of ammonia, a respiratory tract irritant, dust in the poultry house, over-stocking, poor hygiene in the poultry house and a precipitous drop in or increase in the ambient temperature in the chicken house (Barnes and Gross, 1997). Although, colisepticaemia was previously alluded to by the authors, in a study highlighting the impact of infectious bursal disease in Botswana (Binta *et al.*, 1996), this is the first report of *E. coli* infection in broiler chickens without concurrent infections.

The purpose of the present retrospective study, was to investigate the dynamics of an *E. coli* outbreak among broiler chickens on a farm in Botswana. The salient features of the outbreak, predisposing factors as well as the therapeutic and prophylactic intervention were discussed.

MATERIALS AND METHODS

A two-year retrospective study involving 906 batches of broiler chickens submitted to the Animal Health Clinic at the Botswana College of Agriculture (BCA), Sebele was

conducted from January 2001 to December 2002. The number of birds in each outbreak, age groups involved, post-mortem findings, bacterial culture of tissue specimens collected at post-mortem and antimicrobial drug sensitivity of the *E. coli* isolates were recorded.

Swabs taken from various tissue specimens collected at the post-mortem examination of chickens were streaked on blood and MacConkey agar plates. The colonies suspected to be *E. coli* were identified by standard methods (Quinn *et al.*, 1998). Growth on blood agar and evidence of lactose fermentation on the selective medium MacConkey agar, growth on Eosin methylene blue (Levine) agar (Oxoid) and Gram staining reaction followed by biochemical reactions using commercial API 20E reagent kit (Oxoid) were used to confirm the presence of *E. coli*. The API 20E test kits were used and maintained as indicated by the manufacturer.

Swabs of air sac tissues were inoculated into a selective medium for *Mycoplama* species as previously described (Quinn *et al.*, 1998).

Quality control: *Escherichia coli* ATCC 25922 (Oxoid) was used as a reference organism in the confirmatory tests for *E. coli*. The reference organism was stored and maintained according to the manufacturer's instructions.

In vitro antimicrobial drug sensitivity test was performed on confirmed *E. coli* isolates presenting as purple colonies with a black centre and metallic sheen on Levine agar. A previously described standard technique for assessing antimicrobial sensitivity was used (Bauer *et al.*, 1996). The Mueller Hinton agar used in the antimicrobial testing was prepared according to the manufacturer's directions. Using a sterile wire loop, the surfaces of 4 or 5 isolated colonies of a similar morphologic type were transferred to a tube containing 4-5 mL of suitable broth medium. The broth was incubated at 35°C. until its turbidity exceeded that of the McFarland 0.5 barium sulphate standard. Within 5 min of adjusting the density of the inoculum, a sterile cotton swab on an applicator stick was used to streak the dried surface of Mueller-Hinton plates in 3 different planes. The inoculated plates were allowed to remain on flat surfaces. The antimicrobial agents tested included Ampicillin (AP) 10 mg; cephalothin (Kf) 5 µg, colistin sulphate (Co) 25 µg, gentamycin (Gm) 10 µg, Streptomycin (S) 10 µg, Sulphatriad (ST) 200 µg, Tetracycline (T) 25 µg and cotrimoxazole (sulphamethoxazole + trimethoprim). The selection of antimicrobial drugs in the antibiogram was based on their frequency of use in the chicken broiler industry in the country. The discs (Oxoid) used in the antimicrobial drug susceptibility test were stored as recommended by the manufacturer.

RESULTS

Out of a total of 906 cases of diseased chickens submitted for diagnosis at BCA in a two-year period of study, *E. coli* was cultured from one or more of the organs of the dead chickens in 603 (66.6%) of the cases (Table 1). All the isolates of *E. coli* cultured from the swabs possessed biochemical and morphological characteristics of the reference *E. coli* strain (ATCC 25922). The number of cases yielding *E. coli* on bacteriological culture almost trebled over 1 year period from 152-452, the following year.

The dynamics of the infection caused by *E. coli* in chickens is depicted in Fig. 1. Outbreaks caused by *E. coli* were characterised by a high mortality rate for the first 5 days after showing the first signs of illness, peaking on day three. As many as 102 chickens were lost per day. Post-mortem findings included reddish discoloration of carcasses, pericarditis and severe peritonitis. Characteristic off-white cheesy material and pseudo membrane formation on the serosal side of visceral organs were almost pathognomonic of the infection.

The commonest *E. coli*-associated infections diagnosed in this study were air sacculitis and septicaemia. The latter cases accounted for as much as 78.1% (n = 471) of all the *E. coli*-associated cases and were more common in the 1-14 day age group of chicks. Other conditions such as cellulitis, swollen head syndrome, salpingitis, yolk sac infection and enteritis accounted for 14.6% (n = 88). However, in 44 cases (17.3%), the pathological lesions were non-specific.

The monthly distribution of the submissions over a 2 year period showed that more than 40% of the broiler

Table 1: Annual incidence of *Escherichia coli* infection in broilers

Number of cases	Year of study		
	2001	2002	Total
Total number of chickens submitted to BCA clinic	259	649	906
No. of Chickens positive for <i>E. coli</i> infection	151	452	603
Incidence of <i>E. coli</i>	58%	69.9%	66.6%

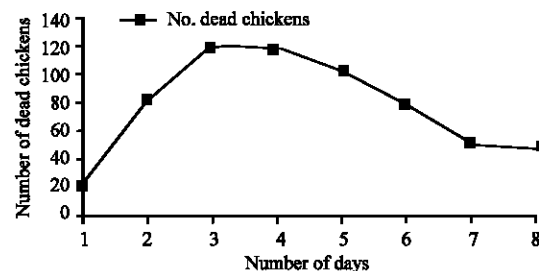


Fig. 1: Dynamics of *Escherichia coli* infections on poultry a farm in Sebele

Table 2: Seasonal incidence of *Escherichia coli* infections in broilers

No. cases	Rainy season				Dry season				Rainy season			
	Jan.	Feb.	Ma.	Apr.	May.	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
No submitted	87	71	72	95	80	61	80	56	97	91	94	47
No. Positive	60	37	38	57	44	44	68	44	65	59	90	35
Prevalence rate	69.0%	52.1%	52.8%	60%	55%	72%	85%	78.4%	66.7%	65%	96%	74%

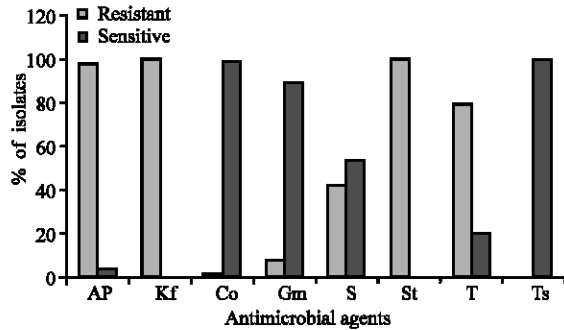


Fig. 2: Antimicrobial drug sensitivity test for *Escherichia coli* isolates

chicken mortality was associated with some *E. coli* infection (Table 1). The seasonal incidence of *E. coli* infections is depicted in Table 2. Apparently, lower infection rates were recorded during the months of February (52.1%), March (52.8%), April (60%) and May (55%), sparing the month of January. This was in contrast to infection rates ranging from 66.7-96% observed from September to December during the rainy season. The rate of infection was significantly higher ($p < 0.05$) in the rainy season ($74.1 \pm 6.9\%$) compared with ($57.0 \pm 8.7\%$) for the winter months with scanty rainfall.

Resistance of *E. coli* isolates was noted for 5 out of 8 antimicrobial drugs used in the antibiograms (Fig. 2). The figure shows that more than 90% of the isolates tested were resistant to ampicillin, cephalothin and sulphatriad. As many as 80% of the isolates were resistant to tetracycline. These isolates were highly responsive to colistin sulphate, gentamycin and cotrimoxazole and to a lesser extent, streptomycin. It was also noted that some *E. coli* isolates exhibited similar antimicrobial sensitivity patterns.

DISCUSSION

In the present study, *E. coli* was recovered from dead broiler chickens at a rate of 66.6% and accounted for as much as 42% of mortality among broilers on this college farm. Organic changes commonly associated with these infections were sacculitis and septicaemia. It is possible that expansion in the broiler chicken population in the area could in part have accounted for the increase in the number deaths caused by *E. coli* infections on the

farm. These findings concur with the previous observations that *E. coli* infections in broiler chickens resulting in air sacculitis and septicaemia are reportedly some of the major threats to poultry farming worldwide (Dozois *et al.*, 1994). As many as 120 chickens were lost in a span of 3 days in this investigation. The results obtained in this study therefore confirm the potential threat *E. coli* infections pose to the fast growing poultry industry in the country.

Although, most of the *E. coli* strains are non-pathogenic, serotypes such as O1, O2, O35, O78 have been associated with extra-intestinal colibacillosis and therefore, potentially pathogenic (Barnes and Gross, 1997). New serotypes including serotype O53 which are highly pathogenic for chicks have recently been identified elsewhere (Blanco *et al.*, 1998).

The seasonal incidence of the condition seemed to suggest an upsurge during the rainy season which is mostly in summer. This finding has been corroborated by other workers (Filali *et al.*, 1988). The bacterium thrives well in poultry houses under wet and humid conditions (Jordan, 1994) which were inevitably prevalent during the summer months when the present study was conducted. This was in contrast to reports from temperate regions where *E. coli* infections flare up during the winter months. Improved hygiene in the chicken house coupled with monitoring of environmental conditions in the chicken house during inclement, weather conditions, failure to dispose of litter from the poultry house coupled with increased humidity in the house may have been conducive for rapid replication of the bacterium. This situation may have been aggravated by poor ventilation.

It is therefore, recommended that thorough cleaning of the poultry house followed by a period of rest before re-stocking may be helpful in preventing pockets of *E. coli* which may be potential foci culminating in outbreaks. Environmental stress induced by the diurnal variation in ambient temperatures is known to exacerbate susceptibility of the chickens to *E. coli* infections (Delicato *et al.*, 2003) sequel to immunodepression.

Increased levels of ammonia, an upper respiratory tract irritant, in the poultry house has also been cited as being contributory to *E. coli* infections. Observations made on the farm in the course of the present study by the authors concur. Perhaps, irritation of the respiratory tract epithelium by ammonia renders the chickens more

susceptible to *E. coli* invasion. It is also possible that interference with respiratory mucosal IgA sequel to general immune compromise induced by stress may render chickens more susceptible to respiratory tract infections.

Although, *E. coli* has previously been found to be secondary to a viral (Binta *et al.*, 1995; Igbokwe *et al.*, 1996) or Mycoplasma agents (Binta *et al.*, 1996), this was not the case in this investigation. Attempts to culture for Mycoplasmal agents yielded negative results. The post-mortem lesions were not suggestive of Gumboro disease infection. Viral infections such as that cause cilia stasis, lymphocytolysis, damage to mucosal barriers and impairment of the mononucleo-phagocytic system may exacerbate colibacillosis in chickens (Igbokwe *et al.*, 1996). No lesions suggestive of a concurrent viral infection were noted in the post-mortems conducted on the chickens in this study.

Due to lack of an effective vaccine against *E. coli*, antimicrobial drug therapy is an important tool in reducing the enormous losses in the poultry industry (Blanco *et al.*, 1997). The high level of resistance of *E. coli* isolates to antimicrobial drugs used in this study seems to reflect extensive use of drugs in the local poultry industry either as feed additives for prophylactic purposes. In some cases therapeutic intervention may not be preceded by antimicrobial sensitivity testing. This may inevitably select for clones of *E. coli* strains with genes for antimicrobial resistance.

Previous studies have indicated high levels of resistance to co-trimoxazole and the new fluoroquinolones which may cause cross resistance with human enteric pathogens thus calling for their prudent use in Veterinary medicine (Amara *et al.*, 1995; Blanco *et al.*, 1997; Zahraei and Farashi, 2006). In this study, the antimicrobial drugs of choice were cotrimoxazole and colistin sulphate. Although, the former drug is able to penetrate the fibrous cheesy pseudomembranes, it may induce resistance or even sensitivity in consumers since it is widely used in human medicine.

The low resistance of *E. coli* isolates to colistin sulphate and streptomycin have been confirmed by other authors (Zahraei and Farashi, 2006). However, since cotrimoxazole and colistin sulphate are not licensed for use in poultry in this country treatment of cases of *E. coli* infections in poultry may become problematical.

Results of a study conducted by Van den Bogaard (2001) strongly indicated that transmission of resistant clones and resistance plasmids of *E. coli* from poultry to humans commonly occurs which may have serious public health repercussions. Indiscriminate use of antibiotics has produced several Multiple Drug Resistant (MDR) enteropathogenic *E. coli* isolates (Ginns *et al.*, 1996).

Other drugs such as calcium fosfomycin (Fernandez *et al.*, 2002) and norfloxacin (Sarkozy *et al.*, 2002) have successfully been used to treat colibacillosis. However, there is need for their legislation for their use in this species in this country prior to incorporation into antimicrobial sensitivity testing discs.

Proposed alternative protection against *E. coli* infection to circumvent the use of antimicrobial agents include the use of DNA containing CpG motifs to engender both local and systemic immunoprotection against the bacterium (Gomis *et al.*, 2003).

Vaccination of chickens using bacterins prepared from local *E. coli* isolates may be worthwhile although attempts in the use of oil-emulsified monovalent *E. coli* bacterins did not confer protection against heterologous challenge (Gyimah *et al.*, 1985). Similarly, the use of genetic variants of *E. coli* to vaccinate broiler chickens against the bacterium may also yield positive results as was reported by other authors (Kwaga *et al.*, 1994).

More recently, the use of *Bacillus subtilis* spores to exclude certain serotypes of *E. coli* in poultry houses has been done with some success (La Regione *et al.*, 2001).

It is also possible to enhance immunocompetence of commercial broilers against *E. coli* and other poultry pathogens by genetic selection as has been done elsewhere (White *et al.*, 1993; Yunis *et al.*, 2002). This would be a long-term strategy. Farmers therefore would not have to use antimicrobial agents since they would only keep chicken lines with established resistance to *E. coli*.

It is therefore recommended that a surveillance program to monitor antimicrobial resistance of to *E. coli* be put in place since transmission of resistant clones and resistance plasmids of *E. coli* from food animals particularly poultry to humans has been documented (Karuiki *et al.*, 1993).

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