Arab Gulf J. scient. Res. **3** (1), pp. 395-406 (1985)

Colisepticaemia on Saudi Arabian Poultry Farms: Strains Biochemical Reactions and Drug Resistance

Elie K. Barbour, N.H. Nabbut, S.W. Hinners and H.M. Al-Nakhli

Animal Production and Health Section, Regional Agriculture and Water Research Center, Ministry of Agriculture and Water, Riyadh, Saudi Arabia

ABSTRACT. Necropsy specimens from chickens on three farms in the Central Province of Saudi Arabia were examined for the presence of colisepticaemia-causing organisms. Seventy eight strains were characterized biochemically and tested for their antimicrobial susceptibilities to eight antimicrobial agents. Most strains exhibited characteristics typical of the Escherichia group with a few exceptions. Thus, 12.8% failed to produce indole; 1.3% utilized citrate and malonate, produced urease and failed to ferment arabinose, maltose and mannitol. Gentamycin and sulfamethoxazole + trimethoprim were the most effective antimicrobials tested. Most of the *E. coli* strains were resistant to two or more drugs in various combinations. The use of sulfachlorpyridazin + trimethoprim for the treatment of colisepticaemia was shown to reduce mortality.

Colisepticaemia is one of the most important diseases prevalent in chickens at 6-10 weeks old. It also occurs in baby chicks and chick embryos (Powell and Finkelstein 1966). Mortality rates usually range from 5-10%, but may reach as high as 50% (Gross 1978). In chickens, virulent *Escherichia coli* strains may cause a primary or secondary infection triggered by an initial mycoplasmal or viral infection (Aycardi and Lafout 1969). Economic losses in such outbreaks are attributed to three factors: mortality rate, lowered weight gain and poor carcass quality.

Biochemical testing of the strains is considered to be significant in these disease conditions (Ewing *et al.* 1974) for differentiating *E. coli* from other intestinal bacteria (Sojka 1965). The emergence of multiple-drug-resistant strains of *E. coli* in poultry has become a major problem (Sato *et al.* 1980). The routine use of subtherapeutic concentrations of antibiotics in feed and water has resulted in the development of large populations of drug-resistant *E. coli* on poultry farms (Linton 1977).

This study deals with the isolation of *E. coli* strains from poultry farms having colisepticaemia problems. Biochemical characterization of the strains, *in vitro* susceptibility testing of the isolates to eight antimicrobial agents and epizootiological investigation of one broiler poultry farm with a long history of colisepticaemia are reported. The emergence of furazolidone-resistant *E. coli* as a result of feeding the drug in low concentrations is discussed. The possibility of reducing mortality in broilers by the use of an alternative drug (sulfachlorpyridazin + trimethoprim) was studied.

Material and Methods

Farm History

Necropsy specimens (livers and hearts) were collected from 31 birds (13 living and 18 freshly dead) on progeny farm 1, while only livers were collected from 26 birds (10 living and 16 freshly dead) on progeny farm 2. On the breeding farm, livers, from 47 freshly dead breeders, 60 inshelled chick embryos and 60-day old chicks were examined. Abnormal mortality rates were not observed on the breeding farm, but the two progeny farms were having recurrent outbreaks. The maximum weekly mortality rates in seven different broods on progeny farm 1 ranged from 8.91 to 34.9%. Since no facial swelling or nasal discharges were observed in the infected birds, mycoplasmosis could be excluded. Records were taken on this same farm of seven broods of reared broiler chicken and their mortalities and nature of antibiotic therapy. One kilogram of furazolidone was added per ton of feed during the two-month growing period. When colisepticaemia occurred, the level of furazolidone was raised to 2.5 kg per ton of feed.

Culturing of Necropsy Specimens

Each of the hearts and livers was seared with a flamed spatula and a loopful of its internal tissue was streaked onto MacConkey agar (Difco). Blood agar was also used for culturing some of the samples, since lesions similar to those produced by *E. coli* may be produced by many other bacteria.

Testing of Water Samples

Water samples were cultured by the multiple tube method (Rand *et al.* 1976). The water samples were collected from the main well of the farm, at different times of about 10 min intervals. This well water is distributed to different reservoirs in the chicken houses.

Biochemical Characterization

Suspected *E. coli* isolates were tested for their ability to ferment five sugars (arabinose, glucose, lactose, maltose and mannitol), production of indole, nitrate

reduction, methyl red and Voges-Proskauer reactions, citrate utilization, gelatin liquefaction, H_2S production, urease production and malonate utilization (Ewing *et al.* 1974).

Antimicrobial Susceptibility Testing

The Bauer-Kirby disk diffusion method (Bauer *et al.* 1966) was used to determine the susceptibility of 78 biochemically confirmed *E. coli* strains. Eight antimicrobial agents commonly used to treat colisepticaemia in poultry were chosen for testing (Table 3).

Results

Chickens from progeny farm 1 showed similar gross lesions, fibrinous pericarditis and hepatitis with serous membrane opacity and gelatinous exudate on the liver and heart surfaces (66.6% of autopsied dead birds and 53.8% of living ones).

Table 1. Sources of 78 *Escherichia coli* strains tested biochemically and for antimicrobial susceptibility.

Source of Strains	Nature of Specimens	Source Index*	No. of Specimens	% of (+ve) Specimens**	No. of Strains Selected to Characterize***
Broiler breeding farm					
Dead breeders	Livers	I	47	40.4	18
Chicken embryos (living)	Livers	II	60	45.0	13
Day-old chicks (living)	Livers	III	60	8.3	2
Progeny farm 1					
One month-old broilers****	Livers	IV	31	64.5	16
	Hearts	V	31	90.3	16
	Well water	VI	10	60.0	5
Progeny farm 2					
One month-old broilers****	Livers	VII	26	30.8	8
Total			265	42.6	78

* Each source is given a different index using roman numbers.
** (+ve) is a symbol for positive.
*** Each strain was recovered from a separate sample.
**** About half the birds examined were clinically ill; remainder had just died.

Some livers, especially those of breeders (23.4%), had only whitish pin head foci of necrosis. Eight percent of autopsied birds on progeny farm 1 had inflamed air sacs and contained yellow caseous material. Seven percent of the living day-old chicks and 14% of the inshelled embryos had caecal casts similar to those found in salmonellosis.

Colisepticaemia was present to various extents on the three farms investigated. Progeny farm 1 had the highest mortality and percentage of positive *E. coli* samples, as observed in 90.3% of hearts examined (Table 1). Profuse pure growth of brick red *E. coli* colonies on MacConkey agar resulted from primary cultures of positive visceral organs, indicating the disease outbreaks were most likely colisepticaemia.

In general, the *E. coli* strains isolated from seven sources (Table 1) on three poultry farms matched biochemically with the known reactions of this organism. A few unusual strains were encountered, such as 12.8% of *E. coli* strains failing to produce indole, the 1.3% utilizing citrate and malonate, producing urease and failing to ferment arabinose, maltose, and mannitol (Table 2).

Table 2. Biochemical characterization of 78 *Escherichia coli* strains encountered on Saudi Arabian poultry farms.

Biochemical Tests	No. of Positive Strains	% of Positive Strains
Fermentation of sugars		
Glucose	78	100.0
Lactose	78	100.0
Arabinose	77	98.7
Maltose	77	98.7
Mannitol	77	98.7
Nitrate reduction	78	100.0
(24 Hr. incubation)		
complete reduction	58	74.4
incomplete reduction	20	2.6
Methyl red reaction	77	98.7
Indole production	70	89.7
Citrate utilization	1	1.3
Malonate utilization	1	1.3
Urease production	1	1.3
Gelatin liquefaction	0	0.0
H_2S production	0	0.0
Voges-Proskauer reaction	0	0.0

The results of *in vitro* susceptibility testing of 78 randomly selected *E. coli* strains to eight antimicrobial agents are shown in Table 3. A wider spectrum of resistance and greater percentages of resistant strains were encountered on the two progeny farms. The *E. coli* isolates from all seven sources showed almost equally high percentages of resistance to streptomycin and sulfathiazole and total susceptibility to gentamycin. The percentages of strains resistant to each antimicrobial agent and their sources are given in Table 4.

Weekly mortality percentages by age groups and broods on progeny farm 1 are reported in Table 5. Furazolidone was given to the seven broods beginning with day one at a low concentration (1 kg/ton of feed) as a preventive measure against recurrent colisepticaemia. The concentration of furazolidone was raised to 2.5 kg/ton of feed whenever outbreaks occurred. In the first five broods, the percentages of mortality in the week following treatment was reduced compared to existing values for the week when treatment was initiated. This reduction ranged from 4.28 to 21.97%. Brood six did not respond to treatment and the mortality in the week following the onset of treatment actually increased. Brood seven, was extensively investigated and 87.5% of the *E. coli* strains recovered from livers were resistant to furazolidone, as shown *by in vitro* testing. Gentamycin or sulfa drug + trimethoprim were prescribed as alternative drugs. Since gentamycin was not available where the farm was located, sulfa chlorpyridazin + trimethoprim was used instead. This alternative drug was added to the water as 1.5 g/liter for a period of three days. The reduction in mortality during the week following treatment was 9.26%.

Table 3. *In vitro* susceptibility of 78 *Escherichia coli* strains encountered on Saudi Arabian poultry farms to 8 antimicrobial agents.

Antimicrobial Agent	Disc Potency	Suscep-tible	%	Inter-mediate	%	Resis-tant	%
Gentamycin	10 mcg	78	100.0	0	0.0	0	0.0
Sulfamethoxazole + Trimethoprim	23.75 mcg + 1.25 mcg	74	94.9	1	1.3	4	5.1
Chloramphenicol	30 mcg	65	83.3	0	0.0	13	16.7
Nitrofurantoin	300 mcg	47	60.3	5	6.4	26	33.3
Furazolidone	100 mcg	44	56.4	3	3.8	31	39.7
Tetracycline	30 mcg	25	32.1	4	5.1	49	62.8
Streptomycin	10 mcg	6	7.7	7	8.9	65	83.3
Sulfathiazole	1 mg	3	3.8	0	0.0	75	96.1

Table 4. Percentage of *E. coli* strains resistant to 8 antimicrobial agents and its relationship to their sources.

Antimicrobial agent	Number of resistant strains	Percentage of resistant E. coli per each indexed source						
		I/18*	II/13	III/2	IV/16	V/16	VI/5	VII/8
Gentamycin	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sulfamethoxazole + trimethoprim	4	0.0	0.0	0.0	12.5	0.0	0.0	25.0
Chloramphenicol	13	0.0	7.7	0.0	37.5	25.0	0.0	25.0
Nitrofurantoin	26	0.0	15.4	0.0	75.0	31.2	20.0	75.0
Furazolidone	31	22.2	15.4	0.0	87.5	37.5	0.0	62.5
Tetracycline	49	33.3	23.1	0.0	31.2	37.5	20.0	100.0
Streptomycin	65	100.0	100.0	100.0	75.0	75.0	0.0	100.0
Sulfathiazole	75	100.0	100.0	100.0	93.7	100.0	60.0	100.0

* Roman number is indexed source and arabic number is number of strains isolated from each source.

Discussion

The isolation of *E. coli* from livers of freshly dead breeders, inshelled embryos and day-old chicks on the breeder farm and from chickens' livers on progeny farms demonstrated the occurrence of colisepticaemia on these farms. The observed symptoms and lesions were typical of this disease (Nasralla and Ismail 1980). In the differential diagnosis, mycoplasmosis could be excluded because of the absence of facial swellings and nasal discharges in the sick birds. It is thought that *E. coli* infection was the primary cause of the outbreaks, especially on progeny farm 1 since *E. coli* colonies were isolated profusely and in pure culture on plates directly streaked from infected organs. The occurrence of highly virulent strains of *E. coli* as the primary cause of colisepticaemia outbreaks was reported by Fagerberg *et al.* (1975).

Biochemical reactions of the 78 *E. coli* strains closely matched with the known group of reactions of this genus with few exceptions. The occurrence of bacterial strains with unusual biochemical reactions has been reported by Weber *et al.* (1981).

The antimicrobial agents tested showed different degrees of effectiveness to the 78 *E. coli* isolates. Gentamycin, sulfamethoxazole + trimethoprim and

chloramphenicol were the three most effective drugs. The emergence of resistant *E. coli* from various avian sources has been under continuous study (Caudry and Stanisich 1979, Dubel *et al.* (1982).

Hellers and Williams (1973) based their epidemiological studies for *E. coli* infections in poultry on resistance patterns of the isolates. The similarities in the percentages of resistance of the *E. coli* isolates, from seven different sources in our investigation, to different antimicrobial agents (Table 4) suggest that such strains originate from one common source. The most likely source for the outbreaks described here is the day-old chick. The various progeny farms in the Kingdom have probably become contaminated by the introduction of infected day-old chicks. The *E. coli* strains recovered from water showed different patterns of resistance to antimicrobial agents than the strains isolated from necropsy specimens on the same farm. This indicates that the water is an unlikely source of the *E. coli* infecting poultry on progeny farm 1.

The intensive use of furazolidone on progeny farm 1 could have resulted in a marked resistance of the *E. coli* isolates (Table 4). The emergence of resistant *E. coli* after prolonged antimicrobial medication of poultry, especially at low concentrations, has been reported by Smith and Tucker (1978) and Braide and Oboegbulem (1980). No work has been reported on the prolonged effect of furazolidone given to poultry at low concentration on the emergence of resistance in *E. coli* strains causing colisepticaemia. It is required by drug manufacturers to determine if the administration of antibacterial drugs at sub-therapeutic levels results in disease that is more difficult or impossible to treat with therapeutic levels of the same drug (Fagerberg *et al.* 1976).

The alternative drug selected from the treatment of brood seven (Table 5), namely, sulfachlorpyridazin + trimethoprim, caused a significant reduction in mortality. This reduction in mortality to normal, due to antibacterial drug, strengthens the evidence of *E. coli* being the primary cause of such outbreaks. Research on this combination for treating colisepticaemia in poultry is continuing (Rosselet *et al.* 1981). This combination has shown a synergistic *in vitro* effect against *E. coli* strains (Amyes 1982) and has been used for treatment of bacterial diseases associated with cancer (Grose *et al.* 1979) and paracoccidiodomycosis in man (Stevens and Vo 1982).

Virulent *E. coli* from chickens could colonize the human gut (Linton *et al.* 1977) but probably would not be as virulent for man. However, such strains could become harmful by transferring their resistance factors to other *E. coli* strains or other bacteria that are virulent for man (Wachsmutch *et al.* 1983).

Testing of newly hatched chicks for potential bacterial pathogens before they are distributed to rearing farms would be most helpful for instituting preventive measures to reduce colisepticaemia and other infections on poultry farms. Evalua-

Table 5. Weekly mortality percentages on progeny farm 1.

Brood No.	Percentage mortality per each week								Reduction in mortality percent**
	1*	2	3	4	5	6	7	8	
1	1.13	1.10	1.13	3.16	11.05	18.90	34.93***	12.96	21.97
2	1.13	1.22	3.00	5.25	8.28	16.73	15.16***	4.93	10.23
3	1.84	1.06	2.08	2.25	1.63	2.90	19.57***	14.23	5.34
4	1.25	1.18	3.81	11.30	17.81	15.13***	10.62	4.37	4.51
5	1.30	1.28	4.62	8.91***	4.63	3.55	4.39	0.33	4.28
6	2.41	0.59	0.46	1.06	3.26	4.93	9.71***	12.25	−2.54
7	0.65	0.55	0.70	0.44	1.44	5.59	10.88****	1.62	9.26

* Furazolidone was added to feed in ratio of 1kg/Ton of feed starting from first day of chicks age, preventive level.

** Reduction in mortality is measured as: % Mortality in treatment week minus % mortality in the week after treatment.

*** Week of treatment with furazolidone, 2.5 kg/Ton of feed.

**** Week of treatment with sulfachlorpyridazine + trimethoprim, 1.5g/liter of water, Cosumix plus®, Ciba-Geigy, Switzerland.

tion of disinfection and hygiene practices on poultry farms and hatcheries for ability to eliminate virulent bacteria is also recommended. Proper intestinal flora in the chicken should be maintained to prevent invasion of the digestive tract by pathogenic bacteria (Adler and Damassa 1980).

References

Adler, H.E. and **Damassa, A.J.** (1980) Effect of ingested lactobacilli on *Salmonella infantis* and *Escherichia coli* and on intestinal flora, pasted vents, and chick growth, *Avian Dis.* **24**: 868-878.

Amyes, S.G.B. (1982) Bactericidal activity of trimethoprim alone and in combination with sulfamethoxazole on susceptible and resistant *Escherichia coli* K-12, *Antimicrob. Ag. Chemother.* **21**: 288-293.

Aycardi, J. and **Lafout, J.P.** (1969) Données recentes sur étiologie et épizootologie de la mycoplasmose du poulet. Conséquences prophylactiques, *Bull. Off. Int. Epizootiol.* **72**: 351-380.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and **Turck, M.** (1966) Antibiotic susceptibility testing by a standardized single disc method, *Am. J. clin. Path.* **45**: 493-496.

Braide, V.P. and **Oboegbulem, S.I.** (1980) Effects of prolonged antimicrobial medication in poultry, *Bull. Anim. Hlth. Prod. Afr.* **28**: 27-34.

Caudry, S.D. and **Stanisich, V.A.** (1979) Incidence of antibiotic resistant *Escherichia coli*

associated with frozen chicken carcasses and characterization of conjugative R plasmids derived from such strains, *Antimicrob. Ag. Chemother.* **16**: 701-709.

Dubel, J.R., Zink, D.L., Kelley, L.M., Naqi, S.A. and **Renshaw, H.W.** (1982) Bacterial antibiotic resistance: Frequency of gentamycin-resistant strains of *Escherichia coli* in the fecal microflora of commercial turkeys, *Am. J. vet. Res.* **43**: 1786-1789.

Ewing, W.H., Davis, B.R. and **Martin, W.J.** (1974) *Biochemical Characterization of Escherichia coli*, U.S. Dept. Health, Education, and Welfare, Public Health Service, CDC, Atlanta, Ga., p. 15.

Fagerberg, D.J., Quarles, C.L., Ranson, J.A. and **Williams, L.P.** (1975) Chick dosage trials with *Escherichia coli, Gen. Ser. Colorado Agric. expl Stn* **948**: 33-35.

Fagerberg, D.J., Quarles, C.L., Ranson, J.A., Williams, R.D., Williams, L.P., Hancock, C.B. and **Seaman, S.L.** (1976) Experimental procedure for testing the effects of low level antibiotic feeding and therapeutic treatment on *Salmonella typhimurium*-var-*copenhagen* infection in broiler chicks, *Poult. Sci.* **55**: 1848-1857.

Grose, W.E., Bodey, G.P. and **Loo, T.L.** (1979) Clinical pharmacology of intravenously administered trimethoprim-sulfamethoxazole, *Antimicrob. Ag. Chemother.* **15**: 447-451.

Gross, W.B. (1978) Colibacillosis, In: **Hofstad, M.S., Calnek, B.W., Helmboldt, C.F., Reid, W.M.** and **Yoder, H.W., Jr. (eds)** *Diseases of Poultry*, 7*th* ed., Iowa State University Press, Ames, pp. 321-330.

Heller, E.D. and **Williams, S.H.** (1973) The incidence of antibiotic resistance and other characteristics amongst *Escherichia coli* strains causing fatal infection in chickens: The utilization of these characteristics to study the epidemiology of the infection, *J. Hyg., Camb.* **71**: 771-781.

Linton, A.H. (1977) Antibiotic resistance: the present situation reviewed, *Vet. Rec.* **100**: 354-360.

Linton, A.H., Howe, K., Bennett, P.M. and **Richmond, M.H.** (1977) The colonization of the human gut by antibiotic resistant *Escherichia coli* from chicken, *J. appl. Bact.* **43**: 465-469.

Nasralla, H.H. and **Ismail, M.R.** (1980) Coli-Bacillosis in chicks in Saudi Arabia, *Trop. Anim. Hlth Prod.* **12**: 15-16.

Powell, C.J., Jr. and **Finkelstein, R.A.** (1966) Virulence of *Escherichia coli* strains for chick embryos, *J. Bact.* **91**: 1410-1417.

Rand, M.C., Greenberg, A.E. and **Taras, M.J.** (1976) *Standard Methods for the Examination of Water and Wastewater*, 14*th* edn., American Public Health Association, Washington, pp. 913-921.

Rigby, C.E., Pettit, J.R., Papp-Vid, G., Spencer, J.L. and **Willis, N.G.** (1981) The isolation of salmonellae, newcastle disease virus and other infectious agents from quarantined imported birds in Canada, *Can. J. comp. Med.* **45**: 366-370.

Rosselet, A., Basler, W., Schluep, J. and **Heim, H.** (1981) Chemotherapeutic activity of the combination of sulfachloropyridazin and trimethoprim against experimental colibacillosis of chickens and piglets and demonstration of the trimethoprim – induced potentiation of sulfachloropyridazin *in vitro* and *in vivo, Schweiz. Arch. Tierheil.* **123**: 401-417.

Sato, A., Tagawa, K., Ike, Y., and **Mitsuhashi, S.** (1980) Drug resistance of *Escherichia coli* strains isolated from chicken, *Jap. J. med. Sci. Biol.* **33**: 185-188.

Smith, H.W. and **Tucker, J.F.** (1978) Oral administration of neomycin to chickens experi-

mentally infected with *Salmonella typhimurium, Vet. Rec.* **102**: 354-356.

Sojka, W.J. (1965) *Escherichia coli in Domestic Animals and Poultry*, Common-Wealth Agricultural Bureaux, Farnham Royal, Bucks, England, pp. 5-17.

Stevenes, D.A. and **Vo, P.T.** (1982) Synergistic interaction of trimethoprim and sulfamethoxazole on *Paracoccidioides brasiliensis, Antimicrob. Ag. Chemother.* **21**: 852-854.

Wachsmutch, K., Deboy, J., Birkness, K., Sack, D. and **Wells, J.** (1983) Genetic transfer of antimicrobial resistance and enterotoxigenicity among *Escherichia coli* strains, *Antimicrob. Ag. Chemother.* **23**: 278-283.

Weber, A., Lembke, C. and **Bertholdt, A.** (1981) Isolierung H_2S – bildender *Escherichia coli* aus urin proben, *Zentbl. Bakt. Mikrobiol. Hyg., I. Abt. Originale A,* **251**: 185-189.

(Received 22/01/1984;
in revised form 09/05/1984/

التسمم الدموى بجراثيم الإيشيريشيا كولاى للدواجن فى مزارع المملكة العربية السعودية : التفاعلات الكيموحيوية والمقاومة للمضادات الحيوية لعثرات الجراثيم

إيلى كميل بربور، نسيم حنا نبوت، سكوت هينرز و حبيب مقبول النخلي

قسم إنتاج وصحة الحيوان - المركز الاقليمى لأبحاث الزراعة والمياه - وزارة الزراعة والمياه - الرياض - المملكة العربية السعودية

تم الفحص الجرثومى لعينات دواجن مشرحة وذلك بغية الكشف عن جراثيم الإيشيريشيا كولاى *Escherichia coli* المسببة للتسمم الدموى فى ثلاثة مزارع بالمنطقه الوسطى للمملكة العربية السعودية. تم تحديد الصفات الكيموحيوية ودراسة الحساسية لثمانية مضادات حيوية وهذا لثمانية وسبعين عثرة.

كانت الصفات الكيموحيوية مطابقة فى معظم الأحوال لمجموعة جراثيم الإيشيريشيا كولاى *Escherichia coli* مع بعض الاستثناءات. لهذا أخفقت ١٢,٨٪ من العثرات فى إنتاج الإندول indole ، استهلكت ١,٣٪ من العثرات مادة السترات citrate ، المالونات malonate وأنتجت إنزيم اليورياز urease ، وأخفقت فى تخمير الأرابينوز arabinose ، المالتوز maltose والمانيتول mannitol .

كانت المضادات الحيوية الاكثر فعالية هى الجنتاميسين gentamycin و السلفامثوكسازول + ترايمثوبريم trimethoprim + Sulfamethoxazole . كانت معظم عثرات الإيشيريشيا كولاى *E. coli* مقاومة لاثنين أو أكثر من المضادات الحيوية فى نماذج مختلفة . إن استعمال السلفاكلوربير يدازين + ترايمثوبريم sulfachlorpyridazin + trimethoprim لمعالجة تفشى مرض التسمم الدموى أظهر مقدرته على انخفاض نسبة الوفيات .