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# INVESTIGATION OF DIFFERENT TYPES OF E. COLI IN POULTRY FARMS IN IRAN *

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**Summary**

The serological and biochemical examinations of 1214 E. coli strains, isolated from clinical materials of diseased chickens are reported. About 1119 strains belong to 12 serological groups, 086, 0111, 026, 02a, 055, 0128.084, 021, 078, 0119,04a, and 0126.

The results of pathogenicity tests using the isolated avian serotypes are described in table 1. A syndrome resembling naturally occurring coli septicaemia was reproduced by the intravenous inoculation of the E. coli - serotypes, most commonly isolated from field cases. The results of in vitro sensitivity tests showed that all serotypes were sensitive to Furazolidone and Nalidixique acid.

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## Materials and Methods

1. E. coli strains:

The 1214 E. coli strains were isolated from the internal organs of chickens which had died of Coli-septicaemia. The strains were collected by preparing cultures on Macconkey agar from the specimens at postmortem. From each plate a few colonies were picked and purified by replating and finally subcultured on Glucose medium, then kept at 4° C. until required.

2. Serological and Biochemical examinations:

a- Biochemical methods:

Eijkmann's test was adapted as the routine test for all the selected - strains, which produced acid and gas in Macconkey-Lactose-Bile broth at 44° C. Such strains were regarded as E. coli.

b. Serological methods:

All 1119 strains were examined serologically using 24 sera, out of which, 11 sera were prepared by using E. coli strains, isolated from poultry at the Microbiology Department of the Razi Institute, and the remaining 13sera were prepared by Wellcome Company. Overnight broth cultures boiled for one hour at 100° C, were tested by tube agglutination test, for determination of 0 antigen as described by Sojka et al 1965 (9). 0 sera were prepared in rabbits using the method described by Sojka et al (9).

K antigen determination: Only those strains isolated from cases of Coli septicaemia which could be placed in one of the 12 groups, were subjected to K antigen dtermination by the slide Agglutination method. A portion of bacterial colony from an overnight culture growth on 5 percent sheep blood agar, was mixed with one drop of saline and one drop of "0" serum. Strains which were smooth in saline and failed to agglutinate with homologous "0" serum were considered to possess K antigen.

Sensitivity tests:

Forty three E. coli strains were tested for in vitro antibiotic sensitivity, performed by the kirbbey Bovuer method (1) using eight disks

containing Nalidixique Acid 30 mg, Penicillin G 10 IU, Neomycin 30 mg, Sulfathiozol 1 mg, Oxytetracycline 30 mg, Chloramphenicol 30 mg, Furazolidone 2 mg, Trimethoprim 1.25 mg plus Sulfamethaoxazole.
Pathogenicity test:

In the first series of pathogenicity test, each strain was cultured in nutrient broth, then overnight culture was washed twice in phosphate buffer saline (PBS) and resuspended in a concentration of $3 \times 10^8$ organisms/ml. These cultures represented 8 serotypes (078, 021, 02a, 055, 084, 026, 086 and 04a) isolated from cases of Coli-septicaemia and one untypeable strain that was isolated from the intestinal contents of a normal fowl. 0.2 ml of each concentration were injected into 5-6 week old chickens intravenously. Experimental chickens were free from Newcastle, S. Pullorum, S. Gallinarum and Mycoplasma organisms.

## Results and Discussion

The results of serological and biochemical examinations of 1214 E. coli strains, isolated from E. coli septicaemia cases indicated that, 1119 strains belong to 12 different "0" sera, as summerised in table 1.

Results of sensitivity test carried out on 43 E. coli strains are shown in table II. These results showed that all strains were sensitive to Furazolidon and Nalidixique acid.

Results of experimental reproduction of disease in 5-6 week old chickens are presented in table III.

A syndrome resembling the naturally occuring Coli-septicaemia, was readily produced by intravenous inoculation of E. coli serotypes isolated from field cases (086, 04a, 026, 084, 055, 02a, 021, and 078). These serotypes were found to be more pathogenic in experimental chicken than were the other serotypes. The one unknown strain, isolated from the intestinal contents of a healthy fowl, faild to produce the disease, when inoculated intravenously into experimental chickens. Death occurred 72 hours following the injection of pathogenic strains. Mortality varied from 85-100 per cent.

E. coli has been known for many years as a pathogenic agent. In spite of the wide use of antibiotic drugs in poultry farms in Iran, colibacillosis plays a very important role in causing heavy losses mostly in young broilers. The result of pathogenicity tests reffered to in this paper, clearly shows that certain E. coli serotypes alone, are capable of producing a syndrome indistinguishable from that observed in field outbreaks of the disease. It is suggested, that some of the field outbreaks of Coli-septicaemia occurring in this country may be caused by E. coli infection alone.

**Results of 0 antigen determination of E. coli**

| 0 groups | No. of strains | Per cent. |
|---|---|---|
| 0111 | 8 | .61 |
| 026 | 14 | 1.20 |
| 02a | 206 | 18.40 |
| 055 | 9 | 0.80 |
| 0128 | 12 | 1.07 |
| 084 | 63 | 5.63 |
| 021 | 193 | 17.24 |
| 078 | 112 | 10 |
| 0119 | 34 | 3.03 |
| 04a | 222 | 19.83 |
| 086 | 228 | 20.37 |
| 0126 | 18 | 1.60 |

Table II.

Results of in vitro sensitivity test of E. coli strains

| Group | No of strains | Sxt | P | N30 | ST | T | C | Fu | Na | |
|---|---|---|---|---|---|---|---|---|---|---|
| 078 | 3 | R | R | S | R | R | R | S | S | |
| 021 | 4 | S | R | R | R | R | R | S | S | |
| 02a | 6 | R | R | R | S | R | R | S | S | |
| 055 | 4 | R | R | R | R | R | S | S | R | |
| 084 | 4 | R | R | R | S | R | S | S | S | |
| 086 | 7 | R | R | R | R | S | R | S | S | |
| 04a | 8 | R | R | R | R | R | R | S | S | |
| 026 | 7 | S | R | R | S | R | R | S | S | |

Table III.

Results of pathogenicity tests

| E. coli Strains | Doses | Chicken's No. | Age of chickens | Route of inoculation. | Number of death. | Pathological signs. |
|---|---|---|---|---|---|---|
| 078 | 0.2 ml | 8 | 5 weeks | I/V | 7 | septicaemia |
| 021 | 0.2 ml | 8 | 5 weeks | I/V | 6 | septicaemia |
| 02a | 0.2 ml | 8 | 5 weeks | I/V | 8 | septicaemia |
| 055 | 0.2 ml | 8 | 5 weeks | I/V | 7 | septicaemia |
| 084 | 0.2 ml | 8 | 5 weeks | I/V | 8 | septicaemia |
| 026 | 0.2 ml | 8 | 5 weeks | I/V | 6 | septicaemia |
| 086 | 0.2 ml | 8 | 5 weeks | I/V | 6 | septicaemia |
| 04a | 0.2 ml | 8 | 5 weeks | I/V | 8 | septicaemia |
| Control | — | 8 | 5 weeks | — | — | No clinical signs. |



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