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## Comparison of a Complement Resistance Test, a Chicken Embryo Lethality Test, and the Chicken Lethality Test for Determining Virulence of Avian *Escherichia coli*

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**SUMMARY.** Results with four pathogenic avian *Escherichia coli* isolates and one avirulent isolate in a complement resistance test, a chicken lethality test, and a chicken embryo lethality test were compared. Results of the complement resistance test with these isolates were highly correlated to results of the chicken lethality test of virulence. The chicken embryo test yielded results that were of a medium positive correlation with the chicken lethality results. The results of the complement resistance and chicken embryo lethality tests were highly correlated.

**RESUMEN.** Comparación de las pruebas resistencia al complemento, mortalidad para embriones de pollo y mortalidad para pollitos, para determinar la virulencia de *Escherichia coli* aviar.

Se compararon los resultados obtenidos con cuatro cepas patógenas y una cepa no patógena de *Escherichia coli* en las pruebas de resistencia al complemento, mortalidad para embriones de pollo y mortalidad para pollitos. Los resultados obtenidos en la prueba de resistencia al complemento se correlacionaron altamente con los resultados de virulencia obtenidos en la prueba de mortalidad para pollitos. Los resultados obtenidos con la prueba de mortalidad para embriones de pollo tuvieron una correlación positiva mediana con los resultados obtenidos en la prueba de mortalidad para pollitos. Los resultados obtenidos con las pruebas de resistencia al complemento y mortalidad para embriones de pollo estuvieron altamente correlacionados.

Virulence of avian *Escherichia coli* isolates is usually assessed by chicken inoculation (4). Such assays may be either unavailable to the laboratory-based researcher or inappropriate for study of genetically modified strains. The purpose of the present study was to identify alternatives to the chicken inoculation model for assessment of microbial virulence. Five avian *E. coli* isolates were compared using a microtiter assay of complement resistance (6), a chicken embryo lethality test (3), and a chicken lethality test (4).

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### MATERIALS AND METHODS

**Test organisms.** Three *E. coli* isolates (V-1, V-2, and V-3) cultured from chickens with systemic infections, one *E. coli* isolate (V-4) from a chicken with omphalitis, and one *E. coli* isolate (AV) known to be avirulent were studied. Isolates were grown in Luria-Bertani broth (5). Cultures for the microtiter test were also grown in PG broth (1% peptone plus 1% glucose) (2). Pure cultures of each isolate were serotyped at Pennsylvania State University, State College, Pa.

**Chicken lethality test.** Twenty 21-day-old commercial broiler chickens per isolate were inoculated intravenously with 10<sup>8</sup> colony-forming units (CFU) (4). Chickens were monitored daily for mortality.

**Complement resistance test.** The *E. coli* used in the present study had been classified as to complement status in a previous study (6). Isolates V-1,

Table 1. Deaths from chicken lethality and embryo lethality tests.

Isolate	Chicken lethality <sup>a</sup>	Embryo lethality <sup>b</sup>	
		10 <sup>2</sup>	10 <sup>3</sup>
V-1	15	18	14
V-2	12	15	16
V-3	8	17	17
V-4	9	2	2
AV	0	1	3

<sup>a</sup>Deaths within 7 days following intravenous inoculation of 10<sup>8</sup> CFU into 20 birds.

<sup>b</sup>Deaths within 4 days following allantoic sac inoculation of 10<sup>2</sup> or 10<sup>3</sup> CFU into 20 embryos per dilution.

V-2, and V-3 are resistant, V-4 is intermediate, and AV is sensitive to the action of complement.

**Chicken embryo lethality test.** Twelve-day-old embryonated eggs were obtained from a commercial hatchery. Overnight cultures of the isolates to be tested were pelleted in a centrifuge (12,000 × g) for 2 minutes and resuspended in phosphate-buffered saline (PBS) to a concentration of 10<sup>2</sup> or 10<sup>3</sup> CFU per 0.10 ml of PBS. This concentration was confirmed by viable plate counts. The 0.1-ml inoculum was injected into the allantoic cavity of each embryo. Twenty embryos per isolate for each dilution were used. Eggs were incubated at 37 C and were candled daily for 4 days to identify dead embryos.

**Statistical analysis.** Relationships between complement class, embryo lethality, and chicken lethality were determined using Pearson's product-moment correlation (1).

RESULTS

Isolates V-1, V-3, V-4, and AV were non-typable, but V-2 was typed O2.

Table 1 shows results of the chicken lethality test and the embryo lethality test. The virulent

isolates killed eight to 15 of the 20 chickens in which they were tested, but the avirulent isolate killed none. Similar results were obtained when the virulence of the isolates was tested in the embryo lethality test. As previously noted, these isolates were categorized by their abilities to resist the bacteriolytic effects of serum complement.

Table 2 shows an intercorrelational matrix constructed from the results of the five isolates in the three assays. Complement resistance was highly correlated to virulence in chickens and in chicken embryos. Embryo and chicken lethality results showed a medium positive correlation.

DISCUSSION

Serotyping produced results reflective of those in the literature. That is, O2 and non-typable classifications are common among virulent avian *E. coli* (4).

Laboratory-based alternatives to the chicken lethality test are desirable because they are easily accessible, are often inexpensive, and provide containment of bacteria at all times. Of the two alternatives examined, the complement resistance test produced results more strongly correlated to the chicken lethality test than did the embryo lethality test for these *E. coli* isolates. These correlations suggest that a complement resistance test may be a good alternative to the use of chicken inoculation and that the results of an embryo test should be interpreted with caution. Perhaps embryo lethality results could be best used to indicate trends in virulence that would be later confirmed in chickens.

Table 2. Intercorrelational matrix of results of three tests for measuring *E. coli* virulence.<sup>a</sup>

Test	Chicken lethality <sup>b</sup>	Complement resistance	Embryo lethality <sup>b</sup>	
			10 <sup>2</sup> CFU	10 <sup>3</sup> CFU
Chicken lethality	—	0.89 <sup>c</sup>	0.77 <sup>d</sup>	0.59 <sup>d</sup>
Complement resistance		—	0.96 <sup>c</sup>	0.88 <sup>c</sup>
Embryo lethality				
10 <sup>2</sup> CFU			—	0.94 <sup>c</sup>
10 <sup>3</sup> CFU				—

<sup>a</sup>*E. coli* isolates V-1, V-2, V-3, V-4, and AV.

<sup>b</sup>Percentages were transformed to arcsines before correlational calculations.

<sup>c</sup>High positive correlation.

<sup>d</sup>Medium positive correlation.

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