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Virulence of *Escherichia coli* Serotypes for Mice[1]

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A study was undertaken to determine whether virulence in mice could be used to assess the pathogenicity of a variety of *Escherichia coli* serotypes. Sixty-one *E. coli* strains isolated from animals, poultry, or humans were serotyped to determine their O, K, and H antigens, and were administered to mice via the intraperitoneal route with and without a mucin adjuvant. The LD_{50} dose was then determined for each serotype. The results indicated that the source of the serotype may be associated with virulence for mice. Serotypes isolated from nonenteric, systemic sources showed a greater virulence for mice inoculated intraperitoneally than did the enteric and the nonenteric, nonsystemic (localized) isolates. It was observed that not all serotypes belonging to a specific serogroup were virulent for mice and that the presence or absence of a K antigen had no effect on the virulence of strains of one serotype.

Escherichia coli serotypes have been associated with diseases of man and animals, especially among the newborn (5, 6, 16). A variety of techniques have been used to assess the pathogenicity of these serotypes (1, 14, 17, 18, 19), virulence in mice being used most extensively. These studies have shown that the pathogenicity may be due to a number of factors involving the host, the agent, and the environment. Investigations involving the agent have indicated that the antigenic composition of the strain is important in determining its pathogenicity. Kauffmann (8), Sjostedt (15), Vahlne (20), and De Witt and Allen (Bacteriol. Proc., p. 119, 1961) have shown that virulence in mice is associated with the presence or absence of the K antigen, and other studies involving the agent have shown that the source, namely the tissue habitat, may be as important as the antigenic composition of the strain in determining pathogenicity for mice (2, 3, 12, 19, 21).

In view of these observations, this study was undertaken to determine the effect of antigenic structure and source of specific *E. coli* serotypes on their virulence for mice.

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MATERIALS AND METHODS

Mice. Swiss-Webster albino mice averaging 25 g were used in all experiments. The animals were housed in groups of five and were provided with a Purina Lab Chow diet and water ad libitum.

Bacteriological methods. Sixty-one strains of *E. coli* of various origins were used in the experiments. With the exception of the human and fish strains, the strains were obtained from diseased animals and poultry submitted for necropsy to the Pennsylvania State University Veterinary Science Diagnostic Laboratory. Isolates from fish were obtained during a water pollution study, and the human strains were obtained from the National Escherichia Center, Atlanta, Ga., and other outside sources. The strains obtained from body tissues and internal fluids of the host were labeled as systemic isolates, those from the intestinal tract as enteric, and those from vaginal tissues, mastitis, salpingitis, urine, or local abscesses as nonsystemic, nonenteric. In some of the postmortem examinations, only the intestine of the host was examined for bacteria, with no attempt to determine whether a septicemia had occurred. Thus, a particular serotype isolated from the intestinal tract may have been present in the system of the host, but its source had to be listed as enteric since no tissues were submitted for bacteriological examination.

Bacterial suspensions for inoculation were prepared from 14-hr Trypticase Soy Broth (BBL) cultures diluted 10-fold from 10^{-1} to 10^{-12} in saline. Amounts of 0.3 ml of the undiluted broth culture and of dilutions 10^{-1} through 10^{-7} were transferred and thoroughly mixed with 2.7 ml of 2% hog gastric mucin (Wilson & Co., Chicago, Ill.; type 1701-w). The final dilutions of each of these bacterial suspensions was then 10^{-1} through 10^{-8} in a 1.8% concentration of mucin. The mucin suspensions and the broth-saline

TABLE 1. *Summary of Escherichia coli serotypes, serological characteristics, source, and LD_{50} for mice arranged according to their O antigen group*

Strain PSU no.	O	K	H	Source	LD_{50} with mucin	LD_{50} without mucin
1	8	41	16	Porcine intestine	5×10^6	6×10^{10}
2	8	41	NM	Porcine intestine	8×10^6	4×10^{10}
3	8	46	NM	Porcine intestine	3×10^7	2×10^{11}
4	8	8	45	Porcine intestine	4×10^6	1×10^{10}
5	8	K·	16	Porcine intestine	2×10^4	1×10^{11}
6	8	K·	19	Fish intestine	8×10^5	3×10^{10}
7	8	46	28	Lamb abscess	4×10^5	1×10^{10}
8	8	K·	NM	Bovine kidney-spleen	1×10^7	1×10^{10}
9	8	K·	9	Bovine intestine	50	5×10^{10}
10	8	43	19	Deer liver-lung	<10	5×10^{10}
11	2a	1	NM	Human blood	<10	8×10^8
12	2	56	1	Human blood	<10	5×10^8
13	2	K·	5	Human blood	<10	1×10^{10}
14	2a	1	NM	Porcine intestine	2×10^7	5×10^{10}
15	2	K—	1	Mastitis	1×10^7	2×10^{11}
16	2a	1	6	Canine urine	5×10^3	5×10^9
17	2a	1	5	Poultry heart	<10	9×10^8
18	78	20	21	Bovine intestine	6×10^6	1×10^{10}
19	78	80	9	Deer intestine	<10	1×10^9
20	78	80	NM	Bovine brain	<10	5×10^9
21	78	80	NM	Bovine spleen	<10	5×10^9
22	78	80	10	Poultry heart	<10	8×10^8
23	109	K—	NM	Porcine intestine	2×10^8	4×10^{10}
24	109	K—	45	Canine liver	3×10^5	3×10^{10}
25	109	K—	21	Bovine vagina	3×10^3	6×10^{10}
26	109	K—	10	Poultry heart	<10	3×10^8
27	109	48	10	Poultry heart	<10	5×10^8
28	115	K—	10	Bovine lung	2×10^3	2×10^{11}
29	115	K·	NM	Bovine lung	11	2×10^{10}
30	115	K·	8	Bovine lung	<10	5×10^9
31	115	K·	NM	Bovine lung	<10	8×10^9
32	115	K·	NM	Porcine edema	<10	4×10^9
33	141	K—	19	Fish intestine	1×10^7	1×10^{11}
34	141	K·	1	Bovine intestine	6×10^6	4×10^{10}
35	141	85	4	Porcine intestine	3×10^3	5×10^{10}
36	141	K·	10	Fish intestine	100	1×10^{10}
37	26	60	11	Porcine intestine	7×10^7	2×10^{10}
38	26	60	NM	Bovine intestine	6×10^7	5×10^{10}
39	26	60	NM	Bovine intestine	5×10^7	1×10^{11}
40	111ab	58	NM	Human intestine	8×10^6	1×10^{11}
41	111ab	58	21	Poultry salpingitis	2×10^7	2×10^{10}
42	111ac	58	4	Poultry pericardial sac	<10	3×10^{11}
43	127a	K·	28	Poultry intestine	5×10^5	2×10^{11}
44	127a	63	NM	Human intestine	2×10^8	1×10^{11}
45	127ab	65	9	Human urine	100	2×10^{10}
46	128ab	67	2	Human intestine	2×10^6	5×10^9
47	128ab	K—	2	Mastitis	5×10^5	3×10^9
48	128ab	K—	35	Porcine kidney	2×10^7	5×10^{11}
49	119	69	4	Porcine intestine	<10	1×10^9
50	119	69	4	Bovine spleen	3×10^3	1×10^{11}
51	138	81	14	Porcine gut edema	50	2×10^9
52	138	81	H·	Bovine intestine	3×10^3	5×10^{10}
53	139	12	NM	Bovine kidney	75	1×10^{10}
54	139	K·	1	Porcine intestine	<10	5×10^9
55	1	K·	NM	Human blood	5×10^3	1×10^9
56	9	35	NM	Bovine intestine	5×10^7	1×10^{11}
57	55	59	1	Human intestine	2×10^3	5×10^{10}
58	101	13	33	Human intestine	6×10^6	2×10^{10}
59	125ab	K—	4	Poultry lung	5×10^3	5×10^{10}
60	126	K·	4	Porcine intestine	1×10^6	1×10^{11}
61	Rough			Human blood	5×10^3	2×10^{11}

suspensions ranging from undiluted through 10^{-5} were administered intraperitoneally in 0.5-ml volumes to the mice. A group of five mice was used for each dilution with and without mucin.

Observations on survival were made for a period of 4 days after inoculation, and the LD_{50} was calculated according to Reed and Muench (13). If 100% mortality was observed for all mucin suspensions of a particular strain, additional mucin suspensions at dilutions of 10^{-9} through 10^{-13} were prepared. These were similarily inoculated into mice; the mortality per dilution was recorded and the LD_{50} was calculated. To determine whether particular lots of mice had any effect on the virulence of the different strains, tests with six strains, of which two were markedly virulent, one virulent, and three nonvirulent, were repeated in a different shipment of mice 4 months later. All six strains showed a comparable virulence in later lots of mice.

Serological identification. The O, K, and H antigens of the *E. coli* strains were identified according to the methods reported in detail by Ewing et al. (4) and Glantz (7). Essentially, this consisted of testing each strain with the standard *E. coli* 145 O antisera, 89 K antisera, 49 H antisera, and reciprocally cross-absorbed serums to determine the antigenic characteristics.

RESULTS

The mouse virulence for each of the 61 *E. coli* serotypes is presented in Table 1. The LD_{50} values obtained without mucin were quite consistent in the number of bacteria required to produce an LD_{50} ($>10^8$) and of little value in separation of the serotypes as to their virulence for mice. The adjuvant effect of mucin is apparent in the reduction of *E. coli* cells required to produce an LD_{50}, especially for the more virulent serotypes (<10).

In Table 2, the serotypes are grouped according to their source, the two main divisions being enteric and nonenteric. The nonenteric group is further divided into systemic and nonsystemic. To make comparisons between the above divisions, four degrees of virulence based on the LD_{50} values obtained with mucin were established for the serotypes. The four degrees are: (i) <10 bacteria per mouse, marked virulence; (ii) 10 to 10^3 bacteria per mouse, virulent; (iii) 10^3 to 10^5 bacteria per mouse, slight virulence; and (iv) $>10^5$ bacteria per mouse, nonvirulent.

Table 2 shows a difference in virulence for mice between the enteric serotypes and the systemic ones. The systemic serotypes showed a high degree of virulence, whereas the enteric ones showed a relative nonvirulence. Of the systemic serotypes, 58% showed a marked virulence, 30% showed some virulence, and 12% were nonvirulent. On the other hand, only 10% of the enteric serotypes showed a marked virulence, 67% were nonvirulent, and 23% showed some degree of virulence. The marked virulence exhibited by 10% of these serotypes may have been due to the method used for their isolation. As previously explained, when these serotypes were isolated from the enteric organs of their hosts, no attempt may have been made to determine whether the bacteria had invaded the host's system. Thus the serotype may have been a systemic one but not recognized as such, and this might account for its marked virulence.

TABLE 2. *Escherichia coli serotypes grouped according to their source*

Enteric		Nonenteric serotypes			
		Systemic		Nonsystemic	
No. of strains	LD_{50}	No. of strains	LD_{50}	No. of strains	LD_{50}
3	<10	14	<10	0	<10
3	10–10^3	2	10–10^3	1	10–10^3
4	10^3–10^5	5	10^3–10^5	2	10^3–10^5
20	$>10^5$	3	$>10^5$	4	$>10^5$
30		24		7	

The nonenteric, nonsystemic serotypes which were isolated from localized infections showed little or no virulence (43%, some virulence; 57%, no virulence).

Table 1 reveals some correlation between virulence and the O antigen group of the serotype. In looking at three particular O antigen groups, 8, 2, and 78, it is apparent that O group 8 has a low degree of virulence and O groups 2 and 78 have a high degree of virulence. However, the source of the serotypes must be considered before any conclusions are drawn. In O group 8, where the serotypes appear to be nonvirulent, it should be noted that seven serotypes were of enteric origins and only one of these exhibited virulence. The majority of the serotypes belonging to O groups 2 and 78 appear to be virulent, but of the 12 tested only 3 were from an enteric source. Thus, the source rather than the O group of the serotype appears to be associated with its virulence for mice. In the case of serotypes belonging to O group 78, the presence of the same O78 and K80 antigen might be an attribute of virulence. Table 3 is included for clarification of the conclusion that virulence is associated with the source of the serotype.

Any conclusions drawn from these results as to the effect of the presence or absence of the K antigen in a particular serotype on the virulence for mice are doubtful. This is because attempts to derive a K minus form from the K plus serotype

TABLE 3. *Comparison of Escherichia coli O groups and source with virulence for mice inoculated intraperitoneally*

Virulence	10 serotypes of O group 8				7 serotypes of O group 2				5 serotypes of O group 78		
	E[a]	S	NS NE	Total	E	S	NS NE	Total	E	S	Total
				%				%			%
Marked.....	0	1	0	10	0	4	0	57	1	3	80
Some.....	2	0	0	20	0	0	1	14	0	0	0
None.....	5	1	1	70	1	0	1	29	1	0	20

[a] E, enteric; S, systemic; NS, nonsystemic; NE, nonenteric.

were not successful. Thus, two serotypes from the same source, one with and the other without the K antigen, required for a valid comparison, were not obtained. However, in the results, O group 109 has four K minus serotypes in which the virulence varies from <10 to $>10^5$ bacteria per mouse. Two serotypes, 109:48:10 and 109:K−:10, identical except for the K antigen, are both from the same source, and both have a marked virulence. These findings indicate that the virulence for mice was independent of the presence or absence of the K antigen for this serotype.

The two strains of serotype 2a:1:NM, one isolated from an enteric source and the other from a systemic one, illustrate that the source may be the key to virulence for mice. The enteric strain is nonvirulent and the systemic one has a marked virulence, although both have the same serological structure.

DISCUSSION

Our results indicated that *E. coli* serotypes of a systemic origin show a high degree of virulence for mice. This observation is in accord with results obtained by other investigations. Waisbren et al. (21) described the clinical aspects of severe nonenteric (systemic) infections in three adult humans caused by strains of *E. coli* which were distinct from known human enteropathogenic types. These three strains were highly virulent for mice via the intraperitoneal route with a mucin adjuvant. Erlandson et al. (2, 3) found that 9 of 10 *E. coli* strains isolated from severe extraintestinal (systemic) infections in adult humans were strikingly virulent for mice by the same route. Four of the strains they found to be highly virulent, PSU-12, PSU-13, PSU-55, and PSU-61, were used in this investigation; two of these, PSU-12 and PSU-13, showed a marked virulence whereas PSU-55 and PSU-61 showed only a slight virulence.

The low degree of virulence exhibited by the enteric serotypes agrees with the findings of others. Erlandson et al. (2, 3) found that only one of six human enteropathogenic strains showed a striking virulence for mice. Turgeon et al. (19) reported that enteropathogenic serotypes of OK antigen groups 127:63(B8), 111:58(B4), 55:59(B5), and 26:60(B6) had little or no virulence for mice via the intracerebral route of inoculation. Thus, it is indicated that *E. coli* strains of enteric origin are for the most part nonvirulent for mice via the intraperitoneal or intracerebral route of inoculation.

The nonenteric, nonsystemic serotypes also exhibited a low degree of virulence. These results combined with those observed for the nonenteric, systemic serotypes and the enteric serotypes lead to the conclusion that the ability of an *E. coli* strain to invade the system of its host appears to be associated with its virulence for mice via the intraperitoneal route. This conclusion is further supported by the behavior of the two strains of serotype 2a:1:NM. Here, the enteric strain was nonvirulent and the systemic isolate of the identical serotype had a marked virulence for mice. The occurrence of virulent and nonvirulent serotypes within the same O group, as demonstrated in this study, is in agreement with findings of Ketyi and Voros (9), who found that there was no relationship between the O antigen of a particular serotype and its virulence for mice. Although the effect of the K antigen on virulence is doubtful because of the failure to derive a K minus serotype from the identical K plus one, the results that were obtained indicate that the K antigen did not have to be present to render this particular serotype virulent for mice. Serotype 109:K−:10 showed a marked virulence for the mice even though it lacked a K antigen.

Further consideration of the relative nonvirulence of *E. coli* serogroups for mice when inoculated by a nonenteric route might include their association with disease in animals and humans. Enteritis due to *E. coli* serogroups 8 in pigs and calves, 26:K60 in humans and calves, and 111ab:K58, 127a, 127ab, 128ab, 55, 125ab, and 126 in humans is well known.

Using the ligated intestinal loop method (enteric route), D et al. (1), Taylor et al. (17, 18),

and Moon et al. (10) showed that serotype was not a factor which determined loop enteropathogenicity. The enteric source or enteropathogenicity of the strain was, however, associated with positive reactions.

A similar result was reported by Mushin and Dubos (11), who found that young albino Swiss mice of the NCS and NCS-D Rockefeller University colonies were highly susceptible to the establishment of intestinal infection with an enteropathogenic strain of *E. coli* O26:K60 administered orally or by stomach tube. The NCS and NCS-D mice colonies were free from detectable *E. coli* cells prior to exposure.

In contrast, the more virulent (for mice) serogroups O2, especially sub O group 2a, 78:K80, 115, and 111ac:K58 are known to produce a septicemia-toxemia, respectively, in humans, calves, and poultry. Serogroups 138, 139, and 141 are commonly associated only with edema disease of swine. Thus, the enhanced virulence of an *E. coli* serotype for mice may be an attribute of its systemic virulence in the host and test animal.

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