

## Occurrence of *Escherichia coli* in Wild Cottontail Rabbits<sup>1</sup>

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Free-ranging cottontail rabbits (*Sylvilagus floridanus*) from two areas in central Pennsylvania were sampled over a 4-year period. Large numbers of coliforms were isolated from the intestinal tracts of these animals; in 136 of the 141 rabbits sampled, *Escherichia coli* was found to be a major component of the alimentary flora. Four serogroups (O7, O77, O73, and O103) were predominant among the isolates and were considered resistant coliflora of this species of cottontail rabbit.

Disease in wildlife is important because of the morbidity and/or mortality it can cause to wildlife species and the resulting effects on their populations. More importantly, wildlife species may act as reservoirs of disease and can be utilized as monitors for the detection of disease and prediction of epidemics (11). Before wildlife species can be utilized as monitors, one must establish the "normal" condition of the animals.

The eastern cottontail rabbit (*Sylvilagus floridanus*) is a common and widely distributed species throughout the United States. This species has a limited home range and is easily handled, sexed, and aged; therefore, it possesses most of the prerequisites of a good indicator species.

Studies on physiology and pathology of cottontail rabbits are numerous; however, little information is available on the prevalence of the enteric bacteria in wild cottontail rabbits. Only one study has documented such information, and the majority of the animals were diseased individuals from a penned colony (12). The objective of this study was to document the prevalence and serological identity of *Escherichia coli* in wild cottontail rabbits. Consequently, once the composition of the resident coliflora in this species has been established, abnormalities can be recognized and changes due to environmental alterations can be detected. Studies to investigate the epidemiological and epizootiological effects of terrestrial wastewater renovation are presently utilizing such an approach.

### MATERIALS AND METHODS

Cottontail rabbits (*S. floridanus*) were live-trapped from wild populations in Centre County,

Pa. From mid-July 1972 to February 1973, 63 rabbits were sampled from State Game Lands 176 (R. Kozlowski, M.S. thesis, The Pennsylvania State University, University Park, Pa., 1974). Beginning in fall 1974 and continuing through spring 1976, rabbits were sampled every fall and spring from two areas, State Game Lands 176 and Rockview Prison. In this 1974 to 1976 period, 50 rabbits from the game lands and 28 from the prison grounds were sampled.

Of the 141 rabbits sampled, total coliform counts were obtained from 40 rabbits and fecal coliform counts were obtained from 30 rabbits. *E. coli* was present in 136 of the 141 rabbits sampled. All of the *E. coli* isolates obtained were serotyped.

Rabbits were sacrificed at Centralized Biological Laboratory, The Pennsylvania State University, on the day of capture. No clinical signs of illness were observed in the rabbits and upon necropsy all internal organs appeared normal. Using an aseptic technique, the intestinal tract was tied off directly below the stomach and removed in situ. The tract was then placed in a sterile jar and refrigerated for no longer than 5 h before processing. An aseptic technique and sterile solutions were always used.

Four sections of the intestine were sampled: (i) duodenum—a 5-inch (12.7-cm) section directly below the stomach, (ii) ileum—a 6-inch (15.2-cm) section above the ileocecal junction, (iii) cecum—a 1-inch (2.54-cm) section 9 inches (22.9 cm) above the vermiform appendix, and (iv) feces—two to three pellets from the posterior inch of the colon. Each of the four sections was tied off at both ends to prevent leakage and removed.

The contents of each section were stripped into physiological saline in a 15-ml graduated centrifuge tube and centrifuged at 4,000 rpm for 4 min. After centrifugation, the wet-packed volume of the intestinal material was measured, and physiological saline was added to give a 1:10 dilution. The samples were then agitated to create a homogeneous solution, and further serial dilutions were made with distilled water.

Three dilutions ( $10^{-2}$ ,  $10^{-4}$ ,  $10^{-6}$ ) were plated in duplicate for each section. Pour plates were made using violet red bile agar (VRB, Baltimore Biological Laboratory). The plates were incubated at 37°C

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overnight for total coliform (TC) and at 44.5°C (water bath) overnight for fecal coliform (FC) counts (1).

After incubation, the plates were counted using a Quebec colony counter, and the results were recorded as the number of colonies per milliliter of wet-packed intestinal material (WPIM). Four morphologically typical *E. coli* colonies from each section were transferred from the VRB plates to stock Trypticase soy agar (BBL) slants. Triple sugar iron agar (TSI, BBL) slants and Simmons citrate agar (BBL) slants were inoculated, and biochemical reactions were noted after 24-h incubations at 37°C and rechecked 4 days later. Organisms producing an acid slant and acid-gas butt on TSI and a negative citrate reaction were considered to be *E. coli*.

The *E. coli* isolates were examined serologically using the methods described by Glantz (5). This consists of a slide agglutination method used for pool, specific, and, when required, cross-absorbed serum tests, and a tube titer for final confirmation of the O, K, and H antigens. *E. coli* standard O group sera 1 to 163 and unclassified OX sera OX1 to OX43 were used for O antigen identification. The K sera used included K1 to K94, and the H sera used to identify the H antigen of motile forms were H1 to H53.

## RESULTS

TC counts (Table 1) were high, with 81% of the samples having counts greater than 100/ml of WPIM. Eighteen of the 30 samples (60%) that had TC counts of less than 100/ml of WPIM were from the duodenum. FC counts were also high, with 75% of the samples having counts greater than 100/ml of WPIM. Again, a large portion (56%) of the samples with FC counts less than 100/ml of WPIM were from the duodenum (Table 2).

The TC and FC counts from the same section of the intestine differed only slightly; however, the counts between sections varied considerably (Fig. 1). The duodenum had the lowest coliform population, with 55% of the TC counts and 66% of the FC counts occurring below 1,000/

TABLE 1. Frequency of occurrence of total coliform counts for 40 cottontail rabbits in various ranges from two areas (1974 to 1976)

Counts <sup>a</sup>	Frequency of occurrence in:			
	Duo- denum	Ileum	Cecum	Feces
<10 <sup>2</sup>	18	8	3	1
10 <sup>2</sup> -10 <sup>3</sup>	4	3	3	3
10 <sup>3</sup> -10 <sup>4</sup>	7	4	12	3
10 <sup>4</sup> -10 <sup>5</sup>	6	3	5	7
10 <sup>5</sup> -10 <sup>6</sup>	2	10	4	1
10 <sup>6</sup> -10 <sup>7</sup>	2	3	9	8
10 <sup>7</sup> -10 <sup>8</sup>	0	7	3	9
>10 <sup>8</sup>	1	2	1	8

<sup>a</sup> Colonies per milliliter of wet-packed intestinal material.

TABLE 2. Frequency of occurrence of fecal coliform counts from 30 cottontail rabbits in various ranges from two areas (1974 to 1976)

Counts <sup>a</sup>	Frequency of occurrence in:			
	Duo- denum	Ileum	Cecum	Feces
<10 <sup>2</sup>	17	6	3	4
10 <sup>2</sup> -10 <sup>3</sup>	3	3	6	2
10 <sup>3</sup> -10 <sup>4</sup>	4	3	8	3
10 <sup>4</sup> -10 <sup>5</sup>	2	0	2	4
10 <sup>5</sup> -10 <sup>6</sup>	3	10	7	3
10 <sup>6</sup> -10 <sup>7</sup>	1	3	2	7
10 <sup>7</sup> -10 <sup>8</sup>	0	4	2	3
>10 <sup>8</sup>	0	1	0	4

<sup>a</sup> Colonies per milliliter of wet-packed intestinal material.

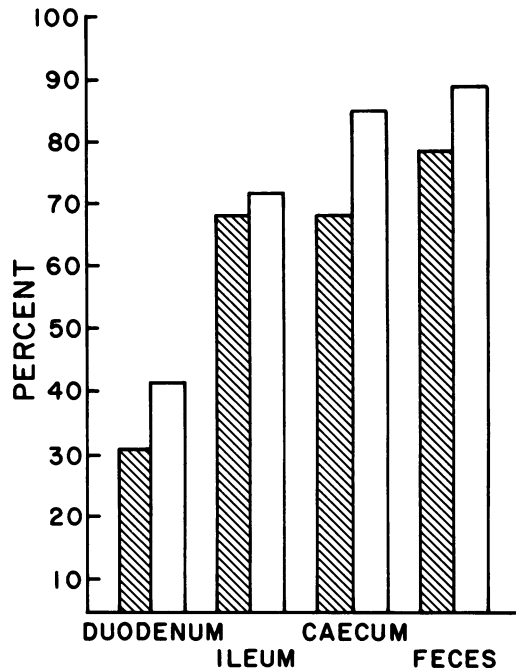


FIG. 1. Percentage of samples from cottontail rabbits having total (striped bar) and fecal coliform (open bar) counts greater than 1,000/ml of wet-packed intestinal material.

ml of WPIM. The feces had the largest amount of coliform, with 90% of the TC samples and 80% of the FC samples having counts greater than 1,000/ml of WPIM. The counts of the ileum and cecum samples were between these two extremes.

*E. coli* was isolated from 96% of the rabbits sampled. These isolates occurred in 33 O groups, 17 of these serogroups being isolated only once (Table 3). Regardless of sample time or area, O7 and O77 were isolated more often

than any other O group, comprising 51% of the total isolates.

The most consistently predominant serogroups that occurred in the rabbits were O7, O77, O73, and O103 (Table 4). Of the 136 rabbits sampled that had *E. coli* present in their intestinal tract, 74% had at least one of these four O groups among the isolates. Although O14 and O15 were more predominant in the 1972 to 1973 sample and O19ab was more predominant in the Rockview sample, O73 and O103 were isolated more consistently throughout this study.

Complete serotyping of the *E. coli* isolates revealed that the K and H antigens for each of the four predominant O groups were identical: O7:K1:H7 or O7:K1:NM, O77:K:H rel 18,

O73:K rel 92:H rel 18, and O103:K:H21 (NM, nonmotile; K-, autoagglutination; rel, related to; K-, negative). In addition, these serotypes were isolated 58% of the time in pure culture (i.e., all isolates from one rabbit were the same serotype). In 60% of the cases, O7 was present in pure culture; O77, O73, and O103 were in pure culture 57, 62, and 50% of the time, respectively. There was no consistency of occurrence of K and H antigens within the less frequently isolated O groups.

## DISCUSSION

Coliform counts presented here indicate the presence of a normal coliflora in the wild cottontail rabbit. This is substantiated by the large number of animals sampled and the consistency of the results between the two areas. Rabbits examined by Smith that had negligible amounts of coliform in the intestinal tract were New Zealand Whites, bred and raised in the laboratory (9). The rabbits examined in the present study were free-ranging cottontails from wild populations. The difference in species associated with a difference in diet, which has an important influence on the alimentary flora (8), may account for the difference in the amount of coliform found in the intestinal tracts of the rabbits in these two studies. In another study (10), adult rabbits (species unknown) were sampled. Although no *E. coli* was found in four rabbits, the other six sampled had *E. coli* counts ranging between  $\log_{10} 2.7$  to  $\log_{10} 6.7$  per g of feces.

A review of the literature revealed one other study of *E. coli* in this species of cottontail rabbit (12). Cottontail rabbits with coliform enteritis, grossly normal cottontails from the laboratory, from an outdoor penned colony and from the wild were examined. These normal cottontails had few *E. coli* which were usually restricted to the lower gut; however, only 10 of the control rabbits were from the wild and can be compared to the rabbits sampled in the pres-

TABLE 3. Occurrence of *Escherichia coli* O groups in cottontail rabbits

O group	Occurrence of O group <sup>a</sup>			
	SLG176 1972- 1973 <sup>b</sup>	SLG176 1974- 1976 <sup>b</sup>	Rock- view 1974- 1976 <sup>b</sup>	Total 1972- 1973 1974- 1976
7	13	29	15	57
9	0 <sup>c</sup>	1	1	2
14	9	0	0	9
15	6	1	1	8
19ab	1	1	3	5
66	0	2	0	2
70	0	1	1	2
73	6	5	2	13
75	1	2	1	4
77	16	13	6	35
88	0	2	2	4
103	5	3	2	10
116	2	0	0	2
121	2	1	0	3
X6	1	2	0	3
X19	0	1	2	3

<sup>a</sup> O groups occurring only once: 2a, 13, 20ab, 23, 26, 43, 51, 53, 87, 105, 108, 139, 144, 150, 151, X1, X3.

<sup>b</sup> Area and years of collection.

<sup>c</sup> 0, Was not isolated.

TABLE 4. Frequency of occurrence of four predominant *Escherichia coli* O groups in cottontail rabbits

O group	Frequency of occurrence				
	SGL176 1974-1976 <sup>a</sup>	Rockview 1974-1976 <sup>a</sup>	Total 1974-1976	SGL176 1972 <sup>a</sup>	Total
O7	29 (62) <sup>b</sup>	15 (54)	44 (59)	13 (21)	57 (42)
O7 and/or 77	38 (81)	18 (64)	56 (75)	29 (48)	85 (63)
O7, 77, and/or 73	40 (85)	18 (64)	58 (77)	35 (57)	93 (68)
O7, 77, 73, and/or 103	42 (89)	19 (68)	61 (81)	40 (66)	101 (74)
Sample size	47	28	75	61	136

<sup>a</sup> Area and years of collection.

<sup>b</sup> Numbers in parentheses indicate percentages.

ent study. The differences in the results between the two studies can be accounted for by a difference in the sampling techniques used. Most of the control rabbits in the Yuill and Hanson study (12) were frozen to simulate the conditions in which the diseased animals were found. This freezing may account for the lack of detectable *E. coli* in the intestinal tract of these animals. Preliminary freezing tests in the present study resulted in a decrease in the coliform counts if freezing was employed. Also, the rabbits in the 1965 study that were not frozen but cultured immediately after death had counts of  $10^5/g$  of intestinal content in the lower intestine.

The total and fecal coliform counts from the four sections of the intestine verify that the coliflora of the cottontail rabbits follows a pattern similar to that found in humans and other animals (2, 3, 7): "... the unaffected small intestine is almost sterile. Only the lower ileum may contain a microbiocenosis, similar to that in the colon but with fewer organisms. Within the colon the microbiocenosis remains constant, and has the same composition as the fecal flora" (7).

Serotyping the *E. coli* isolates obtained from the cottontail rabbits further classifies their natural coliflora. Four predominant serogroups, O7, O77, O73, and O103, can be considered resident strains. A resident strain will persist over a long period of time, whereas transient strains are maintained only a few days or weeks (P. A. M. Guinée, Dissertation in Veterinary Medicine, Rijksuniversiteit Utrecht, Holland, 1963). Since isolates from both sample periods consisted largely of the same four O groups, these O groups may be classified as members of the resident coliflora. Also, indigenous *E. coli* serogroups would appear in a large number of individuals (6). Of 136 rabbits sampled, 74% had one of these four O groups. It is significant that not only four O groups, but four serotypes predominated in the rabbit samples. This predominance of identical serotypes further verifies that the *E. coli* isolated in this study are resident in this species of cottontail rabbits in central Pennsylvania. Antisera prepared for the O77 and O73 isolates indicate that the H antigens of these two serogroups are related and that this may be a new unclassified H antigen.

We are aware of only one other study in which *E. coli* from the intestinal tract of rabbits

was serogrouped (4). Serogroups 2a, 15, and X1 were isolated from rabbits in both studies.

The four consistently predominant O groups isolated from the cottontails do not commonly occur in cattle, sheep, swine, and poultry (5). Thus the frequent isolation of these four O groups from the cottontail rabbits indicate that they are indigenous in this species.

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