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THE ENTERIC BACTERIAL FLORA OF THE INTESTINAL TRACT OF HEALTHY HORSES

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It is well established that the enteric bacteria are responsible as the predominant agent in bacterial abortion of mares and that they are one of the significant secondary invaders in equine infectious anemia. In other diseases of the horse, these bacteria seem to play a role as the causative agent. Though the necessity of studying the enteric bacterial flora in the intestinal tract of healthy horses has been recognized for a long time, no real study has been made because of the lack of a suitable method for classification of the enteric bacteria.

The present paper reports studies on the enteric bacterial flora of the intestinal tract of apparently healthy horses. In this work, the authors have established some common genera of *Enterobacteriaceae* in the feces of healthy horses and also have determined serologically their O groups in *Escherichia coli*.

MATERIALS AND METHODS

Feces from 11 horses were employed. These horses were divided into 3 groups of 1, 8 and 2 animals respectively according to locality.

Immediately after the feces was voided 1g of material was ground with sterile sand; 9 ml of sterile 0.85% saline was added. From this initial dilution, serial dilutions of 10^{-2} , 10^{-4} and 10^{-6} were made; 0.05 ml of each dilution was inoculated on MCCONKEY agar plate and incubated for 24 hours at 37°C. After the incubation the number of colonies on each plate was recorded. From a plate, on which a suitable number of colonies had grown, all colonies were subcultured on nutrient agar slants. Thereafter, all cultures were examined as to their characters by the procedures described in the previous report²⁾. The counts were determined by multiplying the number of the organisms with the reciprocal of the dilution of the plate.

The growth of almost all organisms other than enteric bacteria is inhibited on MCCONKEY agar, so that colonies grown on it may be considered as *Enterobacteriaceae*. *Pseudomonas aeruginosa* and *Bacterium anitratum* are exceptional bacteria that were found to be able to grow on MCCONKEY agar in this study. Accordingly, the total number

of the enteric bacteria was counted by deducting the number of these exceptional organisms from the average count on the MCCONKEY agar.

RESULT

1. Genera of *Enterobacteriaceae*

Enteric bacteria varied greatly in numbers from 21 thousand to 2.6 billion per gram of raw materials with individual feces.

The cultures isolated from the feces were classified as follows: *Escherichia coli*, *E. freundii*, *Klebsiella*, *Cloaca* and *Proteus mirabilis*. Their occurrence in each feces is listed in table 1.

TABLE 1. Number of Colonies of Enteric Bacteria Found in Feces

SPECIES OR GENERA OF BACTERIA FOUND IN FECES	SERIAL NO. OF HORSES										
	1	2	3	4	5	6	7	8	9	10	11
<i>Escherichia coli</i>	247	279	169	119	100	163	193	195	18	31	83
<i>Escherichia freundii</i>	0	0	0	0	0	5	0	0	8	83	0
<i>Klebsiella</i>	3	0	0	0	53	0	0	4	134	0	28
<i>Cloaca</i>	0	0	42	78	51	0	0	0	0	0	21
<i>Proteus mirabilis</i>	0	0	0	0	0	0	0	25	0	0	0
Total	250	279	211	197	204	168	193	224	155	114	132

Escherichia coli: The organisms were isolated from the 11 feces, but their numbers varied widely by individual feces. In comparison with organisms of other genera, *E. coli* gave high counts in 6 of 11 samples; middle counts in 3; and low count in 2. In 2 feces, enteric bacteria other than *E. coli* were not found at all.

Escherichia freundii: They were found in the feces of only 3 horses. In 2 feces, the organisms were extremely inferior in numbers to *E. coli*. On the other hand, in the one remaining feces they were predominant organisms making up about 73% of the total enteric bacteria.

Klebsiella: These organisms were isolated from the feces of 5 horses. They were inferior in numbers to *Escherichia* in 4 feces, whereas they comprised 86% of the enteric bacterial flora in the remaining one sample.

Cloaca: They were isolated from the feces of 4 horses and were always inferior in numbers to other enteric bacteria.

Proteus mirabilis: *Proteus mirabilis* was only once encountered; they made up less than 1% of the total enteric bacterial cultures in this study.

From the above described data it is evident that *Escherichia coli* were the commonest organisms among the intestinal flora of the horses tested. In 2 cases, however, *E. freundii* or *Klebsiella* were predominant organisms therein.

2. Persistence of *Escherichia coli*

Four (A, B, C and D) of the previously noted 11 horses were studied at various intervals for 39~175 days, and *E. coli* cultures from each animal were classified with respect to their serological O groups. For this purpose use was made of 153 sera, of which 19 sera were prepared from cultures isolated from the present animals and the remaining 134 were from KAUFFMANN'S O groups. The former have been distinguished from the latter by the symbols, S 1, S 2, etc., in this report.

From horse A the isolation of cultures from feces at intervals of 5 to 15 days were repeated for more than 6 months. The results are summarized in table 2.

TABLE 2. O Groups of 135 *E. coli* Isolated from Horse A

DAYS AFTER THE BEGINNING OF INVESTI- GATION	NUMBER OF CULTURES ISOLATED	AUTHORS' O GROUPS									OTHERS*	UNGROUPED**
		S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9		
1	8	5	1	2	0	0	0	0	0	0	0	0
8	4	2	0	2	0	0	0	0	0	0	0	0
15	17	7	3	2	1	0	0	0	0	0	4	0
23	11	8	0	2	1	0	0	0	0	0	0	0
30	5	3	0	0	0	2	0	0	0	0	0	0
36	10	5	0	0	0	1	0	0	0	0	4	0
44	3	1	0	0	0	0	1	0	0	0	1	0
50	11	7	0	0	0	0	3	0	0	0	1	0
56	3	0	0	0	0	2	0	0	0	0	1	0
63	10	3	0	0	0	0	5	1	0	0	1	0
70	3	1	0	0	0	0	0	0	0	0	2	0
75	5	2	0	0	0	0	1	2	0	0	0	0
81	6	1	0	0	0	0	4	0	1	0	0	0
88	4	3	0	0	0	0	0	0	0	0	0	1
101	4	1	0	0	1	0	0	0	2	0	0	0
119	5	4	0	0	0	0	1	0	0	0	0	0
126	4	1	0	0	0	0	0	0	0	0	2	1
133	3	0	0	0	0	0	0	0	0	3	0	0
140	3	3	0	0	0	0	0	0	0	0	0	0
151	5	1	0	0	0	0	0	0	0	3	0	1
161	5	4	0	0	0	0	0	0	0	0	1	0
175	6	5	0	0	0	0	0	0	0	1	0	0
Total	135	67	4	8	3	3	17	3	3	7	17	3

* The column headed "others" in table 2 represents a number of cultures which are different from the cultures marked S2, S5, etc., according to their biochemical characteristics.

** "Ungrouped" indicates the cultures ungrouped because of their tendency to agglutinate spontaneously.

From table 2, it is evident that group S1 was found continuously during the entire period of 175 days. This group accounted for about 50% of all cultures examined. Besides O group S1 a number of other O groups, S2, S3, S4, S5, S6, S7, S8 and S9, were found for a short period, but these O groups disappeared from the specimens after several days or weeks and gave place to some other group. On the 44th day after the beginning of the investigation, *Salmonella nagoya* appeared in the feces with a far greater proportion than *E. coli* without any clinical disorders of the animal.

Horse B was studied for only a little over 1 month. In this case all colonies of *E. coli* grown on MCCONKEY agar were picked out and a total of 725 *E. coli* cultures isolated from 4 feces were examined.

TABLE 3. O Group of *E. coli* Isolated from Horse B

DAYS AFTER THE BEGINNING OF INVESTIGATIONS	NUMBER OF CULTURES ISOLATED	O GROUPS				OTHERS			UNGROUPED
		S 10	S 11	S 12	S 13	1	2	3	
1	151	138	13	0	0	0	0	0	0
10	200	191	3	0	0	6	0	0	0
20	200	28	6	166	0	0	0	0	0
39	174	111	2	14	5	0	1	37	4
Total	725	468	24	180	5	6	1	37	4

As shown in table 3, this animal carried two O groups, S10 and S11, at the first examination. S10 was the most predominant population in the feces and accounted for about 65% of all cultures isolated. On the other hand, S11 was very inferior in numbers in every feces examined, yet it was found throughout the entire period. Only the two O groups mentioned above appeared in the first specimens, but 1 to 5 other groups were found thereafter.

Horse C was under observation for about 3 months at intervals of 1 month.

TABLE 4. O Group of *E. coli* Isolated from Horse C

DAYS AFTER THE BEGINNING OF INVESTIGATIONS	NUMBER OF CULTURES ISOLATED	O GROUPS								OTHERS			
		S 1	S 6	S 14	S 15	S 16	S 17	S 18	4	5	6	7	
1	175	2	81	20	3	15	22	5	0	26	0	0	
35	221	0	4	186	0	0	0	1	0	0	0	31	
72	31	0	0	0	0	0	1	0	30	0	0	0	
85	16	0	0	0	0	0	0	0	13	0	3	0	
Total	444	3	84	206	3	16	22	7	43	26	3	31	

At the first examination, 8 groups, S1, S6, S14, S15, S16, S17, S18, etc., were cultivated. Out of them S14 seemed the most predominant O group for 1 month at the beginning of the study. On the examination about 2 months later, the feces failed to yield such groups

as S1, S6, S14, S15, S17 and others and a new group 4 in the columns "others" of table 4, appeared predominantly. That group persisted for the remainder of the observation period. It remains uncertain by what the change of the *E. coli* which was continuously present was caused, but it was not associated surely with clinical disorders.

Horses D and E were cases in which groups *E. freundii* and *Klebsiella*, other than *E. coli*, continued to multiply over a long period. The results are shown in tables 5 and 6.

TABLE 5. Fecal Flora in Horse D

DAYS AFTER THE BEGINNING OF INVESTIGATIONS	NUMBER OF CULTURES ISOLATED	<i>E. FREUNDII</i>		<i>E. COLI</i>	
		Type 3a, 3b, 1c : 8	S20	K28*	Others
1	114	83	21	0	10
58	29	26	0	0	3
89	39	32	0	5	2
Total	182	141	21	5	15

* means KAUFFMANN's O group 28.

In the case of horse D, studied for 3 months at intervals of about 1 month, *E. freundii* type 3a, 3b, 1c : 8 was recovered in all of 3 feces examined. This type accounted for 141 amongst 182 cultures isolated.

TABLE 6. Fecal Flora in Horse E

DAYS AFTER THE BEGINNING OF INVESTIGATIONS	NUMBER OF CULTURES ISOLATED	<i>KLEBSIELLA</i>		<i>E. COLI</i>	
		Type 10*	S3	S21	Others
1	155	134	18	0	3
56	50	50	0	0	0
110	50	32	0	10	8
163	38	21	0	0	17
Total	293	237	18	10	28

* but not completely identical.

Horse E was studied for 5 months. *Klebsiella* type related to capsule 10, but not identical, was continuously demonstrated for over 5 months. It showed a greater proportion than any other culture in every specimen.

In all cases, KAUFFMANN's O group of *E. coli* (O-28) appeared only once.

DISCUSSION

It had been stated by several workers, such as WALLICK and STUART, KAUFFMANN and PERCH, and SEARS et al., that there are 2 kinds of *E. coli* flora

in the human bowel. One of them establishes itself firmly and continues to multiply over a long period; the other is found only in a single specimen or a few successive ones and its total continuance is a few weeks at most. The former was named "residents" by SEARS et al. and the latter "transients".

From the above described examinations on horses, it is evident that the intestinal flora of *E. coli* in stool comprises 2 kinds of organisms, residents and transients, like the case of human beings. After having continued for a certain time, the residents yield their position of dominance to a new resident *E. coli*. It is not clear yet, however, why the microorganisms which have been resident are suddenly displaced by other microbes.

A group found as residents in 1 subject was demonstrated as transients in another. Contrary to expectations, *E. coli* belonging to KAUFFMANN'S O groups 1 to 134 was found rarely. Of total of 1,401 *E. coli* cultures examined, only 5 belonged to KAUFFMANN'S O group 28. This O group was isolated from horse D, as transients, only once.

It is of interest that *E. freundii* and *Klebsiella*, and probably *Cloaca*, are demonstrated as residents in some cases. They may be considered to have the same significance as *E. coli* in the intestinal tract.

SUMMARY

Fecal specimens of 11 horses were studied in order to determine the enteric bacterial flora of the intestinal tract of healthy horse. Then, in 5 of 11 horses the fecal cultivations were made continuously at proper intervals for a long period, and the cultures isolated were examined serologically. The results may be summarized as follows:

1. Enteric bacteria in the intestinal tract of healthy horses examined varied extensively in numbers with individuals. About 2.6 billion bacteria per gram of raw materials were encountered in 1 feces, but only 21 thousand in another.

2. The cultures isolated from feces of 11 horses were classified as *Escherichia coli*, *E. freundii*, *Klebsiella*, *Cloaca* and *Proteus mirabilis*. *E. coli* was the most common bacteria among them, but in some cases *E. freundii* or *Klebsiella* was the dominant bacteria. In the feces of healthy horses the occurrence of *Proteus* is rare, and if it should be present, it is very inferior in numbers.

3. It was ascertained that the *E. coli* flora in the intestinal tract of horses comprised 2 kinds of microorganisms, residents and transients, similar to the human bowel.

4. *E. freundii* and *Klebsiella* were present as residents, over a period of 3 months, in 2 of 5 horses examined.

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