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ENTERIC BACTERIA IN APPARENTLY HEALTHY ANIMALS

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INTRODUCTION

In order to clarify the pathogenic significance of enteric bacteria, it is necessary to ascertain satisfactorily their distribution in normal body, especially in normal intestines. Some of the enteric bacteria such as coliform or paracolon bacteria, as they have long been called, have been considered responsible for various diseases in both animals and human beings. For instance, these bacteria were often accounted in many countries as a causative agent of bacillary abortion of mares and as a secondary invader in equine infectious anemia in Japan. However, it has been difficult to prove the pathogenicity of the bacteria because of the lack of any definite method for their classification.

Since KAUFFMANN published his work with regard to the serological classification of enteric bacteria, it has become easy to demonstrate the pathogenic significance of certain bacteria belonging to the family *Enterobacteriaceae*. However, it seems that nobody has examined the flora of enteric bacteria in healthy animals having in mind the latest findings in enteric bacteriology.

Most of the enteric bacteria are found in the intestines of animals, though the frequency of their occurrence is considered variable according to the species of animals. The mesenteric lymph node seems one of the first portals of infection and a depot of organisms which enter the body through the intestines.

Accordingly, the study of enteric bacterial flora in feces and the mesenteric lymph nodes may give some suggestions to establish the pathogenic significance of enteric bacteria.

MATERIALS AND METHODS

The animals from which the materials were collected are apparently healthy horses, cattle and dogs slaughtered at the Tokyo abattoir. Immediately after the slaughter, the

mesenteric lymph node was removed; then a piece of colon was cut off and its contents were gathered with swab. The material was employed for cultivation aseptically within 2 or 3 hours after collection. A piece of mesenteric lymph node was inoculated on McCONKEY agar plate directly and another piece was put into yeast-casein broth (WATANABE et al.) for enrichment. The enrichment broth was incubated for 24 hours at 37°C; a loopful of broth was smeared on McCONKEY agar plate. The intestinal contents were also streaked on McCONKEY agar plate. After incubation for 24 hours at 37°C, colonies on the McCONKEY agar plate were inspected in detail. From each plate 4 or 5 colonies were taken and inoculated into KLIGLER iron agar, SIM medium, SIMMONS' citrate agar and M R-V P broth. If the colonies on the agar plate were of different appearance, 2 or 3 cultures of each colony type were tested. The growth on the KLIGLER iron agar slant was transferred to the following 12 sorts of media; the fermentative broth containing each arabinose, xylose, rhamnose, sucrose, lactose, adonitol, mannitol, dulcitol, sorbitol, inositol, salicin and glycerol; urea medium and cyanate broth were also used. Indole production was determined after 1-day-incubation at 37°C according to KOVAC's method; the VOGES-PROSKAUER reaction using the BARRIT method after 1-day-incubation at 27°C; the methyl-red reaction after 4-day-incubation at 37 C. The urea and SIMMONS' citrate agar were observed for 4 days and the cyanate broth according to MØLLER for 2 days. Gelatin liquefaction was determined with denatured gelatin according to KOHN. The sugar fermentation was observed for 20 days using the microtechnique of McCREADY et al.

The classification of organisms isolated was made by KAUFFMANN's form.

RESULTS

1. Mesenteric Lymph Nodes

Two hundred and twenty mesenteric lymph nodes each from horses, cattle and dogs were examined. The results are tabulated in tables 1 and 2.

TABLE 1. *Occurrence of Microorganisms in Mesenteric Lymph Nodes*

MICROORGANISMS	SPECIES OF ANIMAL		
	Horses	Cattle	Dogs
Enteric bacteria	107 (53.5 %)	83 (37.7 %)	156 (70.9 %)
Microorganisms other than enteric bacteria	16 (2.4 %)	38 (17.3 %)	55 (25.0 %)
No microorganisms found	97 (44.1 %)	99 (45.0 %)	9 (4.1 %)
Total	220	220	220

Horses: A total of 133 cultures of enteric bacteria were isolated from 107 mesenteric lymph nodes. Ninety-one of them were *E. coli*; 20 were *Cloaca*; 9 *Klebsiella*; 8 *E. freundii*; the remaining 5 were *Salmonella, Arizona* or *P. mirabilis*. From 16 lymph

TABLE 2. *Occurrence of Enteric Bacteria in the Lymph Nodes of the Animals*

GENERA OR SPECIES OF BACTERIA	NUMBER OF CULTURES FROM		
	Horses	Cattle	Dogs
Salmonella	2 (0.9 %)	0	54 (24.1 %)
Arizona	1 (0.5 %)	0	1 (0.5 %)
Escherichia coli	91 (41.3 %)	67 (30.5 %)	118 (53.6 %)
Escherichia freundii	8 (3.6 %)	2 (0.9 %)	1 (0.5 %)
Klebsiella	9 (4.1 %)	0	6 (2.7 %)
Cloaca	20 (9.1 %)	8 (3.6 %)	5 (2.3 %)
Shigella	0	0	1 (0.5 %)
Proteus vulgaris and *mirabilis*	2 (0.9 %)	0	42 (19.1 %)
Proteus morganii	0	0	6 (2.7 %)
Proteus rettgeri	0	0	2 (0.9 %)
Providencia	0	0	6 (2.7 %)

nodes some microorganisms other than enteric bacteria were detected. However, no microorganisms were cultivated from 97 lymph nodes out of the 220 in total.

Cattle: A total of 77 cultures of enteric bacteria were isolated from 83 lymph nodes out of the total 220. Sixty-seven cultures of them were *E. coli*, 8 were *Cloaca* and the remaining 2 were *E. freundii*. Microorganisms other than enteric bacteria were cultivated from 38 and no cultures were obtained from 99 out of 220 lymph nodes respectively.

Dogs: The enteric bacteria were isolated from 156 out of 220 mesenteric lymph nodes. One hundred and eighteen cultures of them were *E. coli*; 54 were *Salmonella*; 42 were *P. vulgaris* and *mirabilis*; 6 were *Klebsiella*; 6 were *P. morganii*; 6 were *Providencia*; 5 were *Cloaca*; 2 were *P. rettgeri*; the remaining 2 were *Arizona* or *Shigella sonnei*. From 55 lymph nodes some other bacteria were isolated and from the remaining 9 no microbes were cultivated.

Escherichia coli was of more frequent occurrence than others in the above-noted animal species. Of 452 cultures of enteric bacteria isolated, 276 were identified as *E. coli*.

Salmonella and *P. vulgaris* and *mirabilis* were common next to *E. coli* in dogs. These groups of enteric bacteria were found in 54 and 42 respectively of 220 mesenteric lymph nodes; they occurred in over 20% of all the cultures isolated. In horses and cattle, however, they were encountered rarely.

Cloaca and *Escherichia freundii* were isolated from each animal species with low frequency. On the other hand, *Proteus morganii*, *P. rettgeri* and *Providencia* were found only in dogs.

On histopathological examination, the occurrence of some histological changes,

such as hemorrhages, sinus catarrh and others, was demonstrated in the lymph nodes from which the enteric bacteria were isolated by the direct cultivation. However, the mesenterics in which the enteric bacteria were found only after the enrichment, revealed no evidence of histological changes.

2. Intestinal Contents

Intestinal contents from each 220 horses, cattle and dogs were examined in the same manner as above described. The results are given in table 3.

TABLE 3. *Occurrence of Enteric Bacteria in 220 Feces of the Animals*

GENERA OR SPECIES OF BACTERIA	NUMBER OF CULTURES FROM		
	Horses	Cattle	Dogs
Salmonella	0	0	21 (9.5 %)
Escherichia coli	213 (96.6 %)	219 (99.9 %)	220(100.0 %)
Escherichia freundii	23 (10.5 %)	11 (5.0 %)	24 (10.9 %)
Klebsiella	31 (14.1 %)	14 (6.4 %)	31 (18.1 %)
Cloaca	43 (15.0 %)	20 (9.1 %)	23 (10.5 %)
Proteus vulgaris and *mirabilis*	2 (0.9 %)	2 (0.9 %)	74 (33.6 %)
Proteus morganii	0	0	2 (0.9 %)
Providencia	0	0	2 (0.9 %)

Horses: A total of 312 cultures of enteric bacteria including 213 cultures of *E. coli*, 23 of *E. freundii*, 31 of *Klebsiella*, 43 of *Cloaca* and 2 of *P. mirabilis* were isolated. *Salmonella*, *P. morganii*, *P. rettgeri* and *Providencia* were not found at all.

Cattle: From the intestinal contents of cattle a total of 264 cultures of enteric bacteria including 219 cultures of *E. coli*, 11 of *E. freundii*, 14 of *Klebsiella*, 20 of *Cloaca* and 2 of *P. mirabilis* were cultivated. However, *Salmonella*, *P. morganii*, *P. rettgeri* and *Providencia* were not obtained.

Dogs: A total of 397 cultures of enteric bacteria comprising 220 cultures of *E. coli*, 21 of *Salmonella*, 24 of *E. freundii*, 31 of *Klebsiella*, 23 of *Cloaca*, 74 of *P. vulgaris* and *mirabilis*, 2 of *P. morganii* and 2 of *Providencia* were isolated. However, *P. rettgeri* was not found.

From the above-noted results, it is obvious that the kind of the various genera of enteric bacteria found in lymph nodes is closely similar to that of feces. However, the percentage of each genus in lymph nodes differs widely from that in feces.

In most cases, colonies grown on a McCONKEY agar plate were composed of organisms belonging to 2 or 3 genera with the colonies of *E. coli* predominating over the others. In some instances, however, colonies of *E. freundii*, *Klebsiella* or *Cloaca* outnumbered those of *E. coli* and some intestinal contents contained exclusively 1 group of enteric bacteria other than *Escherichia coli*.

Discussion

As mentioned above, *E. coli* is the organism most commonly encountered in the mesenteric lymph nodes as well as in the feces in every animal species here considered. On the other hand, the frequency of occurrence of the genera other than *E. coli* seems to be characteristic to the animal species.

Cloaca and *Klebsiella* were found rather frequently in horses and cattle but *P. vulgaris* and *mirabilis* and *Salmonella* infrequently. Moreover, *P. morganii*, *P. rettgeri* and *Providencia* were not found at all in horses and cattle. On the contrary, *P. vulgaris* and *mirabilis*, and *Salmonella* were encountered more frequently in dogs, while *P. morganii*, *P. rettgeri* and *Providencia* were not encountered so frequently. The frequency of *Cloaca* and *Klebsiella* in dogs was lower than in horses.

It is not clear exactly what the above-described results mean, however, the genus which is found frequently in the mesenteric lymph nodes is also isolated often from pathological materials. For instance, *Cloaca* group is of frequent occurrence in pathological materials from horses; the present workers frequently isolated *Cloaca* cultures from aborted equine fetuses free from *Salmonella abortus equi* and from the horses suffering from equine infectious anemia in Japan (unpublished data). *P. vulgaris* and *mirabilis* should not always be considered responsible for diarrhea of the dog in every case when these organisms are recovered from diarrheal stool of the animal. On the contrary, when many colonies of *P. vulgaris* and *mirabilis* are observed in the culture from stool of a sick horse, these organisms may be accountable for the sickness. This is true because *P. vulgaris* and *mirabilis* is a common intestinal population of the dog, but not of the horse.

As Galton et al. and others have reported, *Salmonella* was found more frequently in dogs, but its pathogenic significance in this animal has to be studied in the future.

It is apparent that genus *Arizona* is spreading gradually over Japan, since the organisms were isolated from mesenteric lymph nodes of horses and dogs, and more recently of pigs, although the genus was never found in the great number of materials examined up to several years ago.

Shigella sonnei was isolated only once from a mesenteric lymph node of the dog, however, pathogenic significance in the animal cannot now be discussed on the basis of such scanty data.

There was no feces from which the enteric bacteria were not isolated, though remarkable differences existed between herbivores and carnivores in total number of the organism. For instance, the authors often encountered materials from which only a few colonies developed in spite of inoculation with a considerable

amount of feces of herbivore on a McCONKEY agar plate. In general, the enteric bacteria in feces of the horse and the cattle are less in number than in those of the dog. The fact that few enteric bacteria are found in feces of herbivores seems due to the antagonism existing in the intestines between the bacteria and other organisms, as described by WRAMBY.

For the present, it may not be asserted whether the enteric bacteria isolated from mesenteric lymph nodes, as well as the organisms from feces, are to be regarded as normal flora or not. In addition, the mesenteric lymph node is one of the most regenerative organs, so that the absence of pathological changes in the lymph node does not always deny the existence of some infections.

In dogs, *Salmonella* were found in 24.1% in mesenteric lymph nodes, but in 9.5% in feces. From such data, the mesenteric lymph node may be considered as the depot of the pathogenic enteric bacteria. However, there is no proof that the organisms of genera other than *Salmonelleae* in mesenteric lymph node differ from normal intestinal flora. In any case, comparative studies, in future, on the biochemical and serological behaviours of the enteric bacteria collected from the normal materials, mesenteric lymph nodes, feces and the pathological matters will resolve the question whether essential differences exist or not among these bacteria derived from various sources.

SUMMARY

Enteric bacteria from the mesenteric lymph nodes and intestinal contents of normal horse, cattle and dog have been examined.

Escherichia coli was found to be the commonest organism in the mesenteric lympe nodes as well as in feces of every animal species investigated. It seems sure, however, that the detection frequency of the genera other than *E. coli* is characterstic to the animal species. In the horse, genera *Cloaca* and *Klebsiella* were found rather frequently, however, *Salmonella* and *Proteus* were found rarely. On the other hand, in dogs, genera *Salmonella* and *Proteus* were often isolated, but *Cloaca* was infrequently.

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