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SULPHATE - REDUCING BACTERIA IN CANINE FAECES

K. Tayfun ÇARLI*

SUMMARY

Ten (33.3 %) sulphate-reducing bacteria (SRB) were isolated from 30 canine faeces. Of 10 isolates examined, all were assigned to the genus Desulfovibrio. The number of Desulfovibrio sp. in canine faeces ranged from 1 x 10^3 to 6x 10^{10} bacteria g^{-1} Postgate's Medium E seemed to be more efficient than Postgate's Medium B and ATCC Culture Medium 1249 for isolation from canine faeces.

Keywords: SRB, Desulfovibrio, canine faeces.

ÖZET

Köpek Dışkılarında Sülfat-İndirgeyen Bakteriler

Otuz adet köpek dışkısından 10 (% 33.3) sülfat-indirgeyen bakteri (SİB) suşu izole edildi. İncelenen 10 izolatın hepsinin Desulfovibrio genusunda oldukları belirlendi. Köpek dışkılarında Desulfovibrio sayısı 1 x 10^3- 6 x 10^{10} bakteri g^{-1} arasındaydı. Köpek dışkılarından izolasyonda Postgate's Medium E'nin, Postgate's Medium B ve ATCC Culture Medium 1249'dan daha etkin olduğu görüldü.

Anahtar Kelimeler: SİB, Desulfovibrio, köpek dışkısı.

* *Doç. Dr.; U.Ü. Vet. Fak. Mikrobiyoloji ABD, Bursa-Türkiye.*

INTRODUCTION

A role of sulphate-reducing bacteria (SRB), a group of obligately anaerobic organisms capable of reducing inorganic sulphate to hydrogen sulfide, in the possible pathogenesis of ulceratif colitis (UC) in humans has been proposed in a series of reports[1,2,3,4].

It is noteworthy that *Desulfovibrio* species which seems to be most dominant SRB in faeces, share 87.5-91 % homology with the 16S rRNA sequence of a curved intraepithelial bacillus (previously implicated as intracellular *Campylobacter*-like organism (ICLO)) consistently associated with intestinal lesions in animals with proliferatif bowel disease[5,6]. Therefore, the potential significance of these animal models with colitis is now emerging following identification in rumenal[7,8,9] and colonic[10,11,12,13,14] contents of SRB, in particular *Desulfovibrio* sp.

The purpose of this study is to determine presence and average number of SRB in canine faeces, and to compare some media for the isolation.

MATERIALS and METHODS

Faeces: Thirty faecal samples were collected into screw-capped sterile test tubes from healthy and enteric dogs in Veterinary Faculty Clinics, Uludag University, Bursa and Bursa Nilüfer Municipality Veterinary Division Directorate. The samples were used within 4 hours without any storage and delay for isolation.

Isolation: One gram of samples from each dog was homogenized with 9 ml physiological saline (PS) (0.9 %) solution. For isolation, three different types of media were used and they were all prepared at same time when the samples were collected.

Postgate's Medium B[1]: Nine ml of this medium was added into six screw-capped tubes and sterilized in autoclave. After autoclaving, the media in tubes were hold molten 40-45°C. Then, ten-fold dilutions were done of the samples and the dilutions were incubated at 30°C under anaerobic conditions. After 4 or 5 days well-seperated black colonies were considered to be SRB.

Postgate's Medium E[1]: One ml of homogenized sample was added into 9 ml fresh PS to give second 10^{-2} dilution, and so on down to about 10^{-6} Each dilution, in 1 ml lots, was added to test tubes with sterile pippet, and 6 ml of medium E was added. The contents were mixed by inverting the tubes and, once the agar set, an extra 1 ml of agar is added to exclude air from the inoculated portion. Tubes were incubated at 30°C for 4 or 5 days and the black colonies were counted.

ATCC Culture Medium 1249: This medium was prepared as described in the webpage of American Type Culture Collection on internet, http://www.atcc.org/cgibin/Sfgate?langua...20314706%20%2fpub%2ftextfiles %ZflVIEDIA. TXT. Briefly, Component I, Component II with 15 % agar (Difco 0140-01, Detroit, Michigan) and Component III were prepared seperately and autoclaved. Under aseptic conditions, these three solutions were mixed, and 9 ml of the mixture, which had contained 3 % final agar concentration, was added into six screw-capped sterile tubes. When medium temperature came down to 45°C, 0.18 ml of filter-sterilized 5 % ferrous ammonium sulphate was added to each tube. Finally, faecal homogenates were diluted ten-fold and they were allowed to incubation at 30°C for 3 weeks. After incubation, well-seperated black colonies were counted.

Identification: To identify some isolates, single colonies were removed from the highest dilution of agar medium from each positive sample and inoculated into liquid medium B. Pure cultures were isolated by transfer of single colonies through solid and liquid media. The bacteria were assigned to specific genera on the basis of morphology, motility and desulfoviridin production and spore formation[1,15].

RESULTS

The presence of sulphate-reducing bacteria were shown in this study. The number of SRB in canine faeces ranged from $1X10^3$ - $6X10^{10}$ bacteria g^{-1} (Table 1). Of 30 dogs examined 10 (33.3 %) were found to harbour SRB in their faeces by the use of Postgate's medium E for isolation, but these ratio were 20 % by Postgate's Medium B and 13.3% by ATCC Culture Medium 1249. No difference was observed between healty and enteric dogs. Also number of SRB did not show any variability depending on the ages of dogs examined.

In the morphological examinations, the isolates were curved, sometimes "s" shaped rods, Gram negative, non-sporeforming. They were all found to be mesophilic and obligately-anaerobic. All isolates were determined to produce desulfoviridin. Also, they were all observed to be very motile. On the basis of these criteria mentioned above, all the isolates were identified to belong to the genus *Desulfovibrio.*

In addition to the genus *Desulfovibrio* colonies, bacterial colonies containing Gram-positive sporeforming, non-sporeforming rods were also observed in the same culture medium. These colonies were generally small, pinpoint and white in colour. The other bacterial colony type found in the same culture with the genus *Desulfovibrio* was seen a small and non-progressive and black in colour. These small blackish colonies had also non-sporeforming, curved and Gram negative bacteria, similar to the genus *Desulfovibrio*, but they did not give desulfoviridin reaction.

DISCUSSION

The cause of ulcerative colitis (UC) is unknown but it is likely to depend on an interaction between genetic factors, which may determine the immune response or the expression of enzymes that control intracellular metabolism, and environmental factors such as diet and the nature of the bacterial flora[16]. SRB may be an important factor with respect to the health of host. They may be normally found in colonic flora in humans and some animals[7.11.12.13.14]. Isolation of SRB together with *Desulfovibrio* sp from canine faeces sample in the present study is the first report in the literature.

Carriage of SRB has been shown to be 96% in colitis patients, 50% in health persons by Florin et. al[4], to be 66% in healty persons by Gibson et al[3]. SRB isolated from faeces of humans[2.11.12] and cattle[14] and from bovine[9] and ovine"[7.8] ruminal fluid have been assigned to the genus Desulfovibrio sp.

In the present study, we found the carriage of *Desulfovibrio* as 33% and the some other bacteria other than *Desulfovibrio*. This indicate that the dogs have a difference from human and cattle for SRB carriage in their faeces.

Severel culture media have been described and used for isolation of SRB in different studies. In our study, Postgate's medium E seems to be more satisfactory medium among the three media used. The medium E is also advised for *Desulfovibrio* sp. isolation from environmental samples by Postgate[1].

In conclusion, this paper demonstrates for the first time that SRB belonging to the genus *Desulfovibrio* are present in canine faeces, although the dogs have been determined to carry the SRB in their faeces lower than humans and cattle.

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Table: I

Samples, Media and Numbers of Sulphate - Reducing Bacteria

Sample			Media		
Sex	Age	H.C.	P.M.B. 1249	P.M.E.	ATCC
M	3 years	Healthy	10×10^2	1.0×10^4	1×10^4
F	1.5 months	Enteric	10×10^3	22×10^4	N.G.
F	4 months	Healthy	20×10^3	20×10^4	16×10^4
F	1 years	Healthy	N.G.	N.G.	N.G.
M	6 months	Healthy	N.G.	N.G.	N.G.
F	2 years	Healthy	N.G.	N.G.	N.G.
M	1.5 months	Healthy	N.G.	N.G.	N.G.
M	1.5 months	Healthy	N.G.	N.G.	N.G.
F	4 years	Healthy	N.G.	N.G.	N.G.
F	10 months	Healthy	N.G.	22.5×10^3	N.G.
M	2 months	Healthy	N.G.	N.G.	N.G.
F	5 years	Healthy	N.G.	N.G.	N.G.
M	1.5 years	Healthy	N.G.	N.G.	N.G.
M	8 months	Healthy	N.G.	30×10^5	N.G.
F	6 months	Healthy	20×10^3	40×10^6	30×10^4
M	2.5 years	Healthy	N.G.	N.G.	N.G.
M	1 years	Enteric	N.G.	N.G.	N.G.
F	4 years	Enteric	10×10^3	13.5×10^4	N.G.
F	3 years	Enteric	20×10^4	60×10^9	N.G.
F	4 years	Healthy	N.G.	10×10^4	N.G.
M	3 years	Healthy	N.G.	N.G.	N.G.
F	4 years	Enteric	N.G.	N.G.	N.G.
M	5 years	Enteric	N.G.	N.G.	N.G.
F	4 years	Enteric	N.G.	N.G.	N.G.
M	8 years	Enteric	N.G.	N.G.	N.G.
M	4 years	Healthy	N.G.	N.G.	N.G.
M	3 years	Healthy	N.G.	N.G.	10×10^3
M	8 years	Healthy	N.G	30×10^3	N.G.
F	4 years	Healthy	N.G.	N.G.	N.G.
F	4 years	Healthy	N.G.	N.G.	N.G.

M.: Male, F.: Female, H.C.: Health Condition, P.M.B.: Postgate's Medium B, P.M.E.: Postgate's Medium E, ATCC 1249: ATCC Culture Medium 1249, N.G.: No Growth