Jpn. J. Vet. Res., **28**, 86-94 (1980)

OCCURRENCE OF ENTEROTOXIGENIC STAPHYLOCOCCI IN HOUSEHOLD AND LABORATORY DOGS

Yoshifumi KAJI*[1] and Eiichi KATO*[2]

Department of Public Health
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

(Received for publication, March 18, 1980)

Enterotoxigenic staphylococci (ES) were isolated from the exterior body sites including the suppurative lesions of 15 (13.0%) out of 115 household dogs. Of the total 26 toxigenic isolates from the household dogs, 21 belonged to biotype E (canine origin, according to Baird-Parker's classification modified by HÁJEK & MARŠÁLEK) and produced enterotoxins C (18 isolates) and A (3 isolates), 4 belonged to biotype A (human origin according to the classification) and produced enterotoxins A (3 isolates) and D (1 isolate), and the remaining one belonged to biotype B (classified as swine or poultry origin) and produced enterotoxin C. ES were also isolated from the exterior body sites of 2 out of 9 laboratory dogs which were examined monthly over one year. All of the 6 toxigenic isolates from the laboratory dogs belonged to biotype E and produced enterotoxins A+C (3 isolates) and C (3 isolates). ES in household dogs were considered to be important because they produced enterotoxins A, C, and D, which frequently cause food poisoning in humans.

INTRODUCTION

Enterotoxigenic staphylococci (ES) were isolated from the cecums and rectums of 26 (5.8%) out of 451 stray dogs[7]. Of the total 30 enterotoxigenic isolates, 14 belonged to biotype E (canine origin according to Baird-Parker's classification modified by HÁJEK & MARŠÁLEK)[5] and produced enterotoxin C exclusively, which was the first evidence of ES of canine origin.

The present paper deals with the enterotoxigenicity and biotypes of canine staphylococci isolated from household and laboratory dogs.

MATERIALS AND METHODS

Specimens One hundred and fifteen household dogs, which were out-patients at the Veterinary Hospital of the Faculty of Veterinary Medicine, Hokkaido University, Sapporo,

*1 Present address: Yokohama Food Sanitation Inspector's Office, the Ministry of Health and Welfare, Yokohama 231, Japan

*2 Present address: Department of Veterinary Public Health, Faculty of Agriculture, Tottori University, Koyama, Tottori 680, Japan

were examined between July and November, 1977. Swabs were obtained from the nasal cavity, external auditory canal, dorsal skin, and anus, and sometimes from the exterior suppurative lesions in each dog. Swabs were also taken monthly from the same sites in 9 laboratory dogs (beagles), which were kept at the Department of Surgery of the same Faculty, during the period from September, 1977 to August, 1978.

Isolation of coagulase-positive staphylococci The isolation procedure followed a method described previously[7]. All swabs from the nasal cavity, external auditory canal, dorsal skin, and suppurative lesions were streaked on a blood agar plate; the swabs taken from the anus were streaked on a staphylococcus medium (Nissui). The blood agar plates were incubated at 37°C for 24 hours, and the staphylococcus medium plates were incubated at 37°C for 40 hours. Each staphylococcus-like colony (several colonies, including yellow and white, were examined from each sample) was transferred to a sheep blood agar plate. From the colonies grown on the blood agar plate, a representative yellow or white colony (or both) was selected and tested for the production of coagulase using rabbit plasma. One coagulase-positive colony (yellow, white, or both) from each specimen was selected for further study. The cultures were stored in trypticase soy agar (BBL) at room temperature.

Enterotoxin typing The staphylococcal isolates were tested for their ability to produce enterotoxins A, B, C, D, and E. Production of the enterotoxins followed the method described previously[6]. The enterotoxins in a culture supernatant and in a concentrated culture supernatant (40 times) produced by the staphylococcal isolates were identified by the microslide double gel-diffusion technique, as described by KATO et al. (1978). The lowest level of enterotoxins detectable by the test was 1.0 μg/ml of the reference enterotoxins. The reference enterotoxins and their corresponding antisera were supplied by M. S. BERGDOLL of the Food Research Institute, the University of Wisconsin, Madison, U. S. A.

Coagulase test The coagulation of fresh rabbit, human, and bovine plasmas was tested in tubes according to the method described by KATO & KUME (1980). Readings were made after 24, 48, and 72 hours of maintenance at room temperature, as well as after 1, 2, 3, and 4 hours of incubation at 37°C. A solid and fibrinous coagulum was evaluated as being positive.

Production of fibrinolysin The fibrinolytic activity was tested in a medium containing 20 % human plasma. The test procedure followed the method described by SATO et al. (1972).

Production of haemolysin The production of alpha- and beta-haemolysins was determined by using washed rabbit and sheep erythrocytes, which were added to nutrient agar (Eiken) to obtain a final concentration of 3 %. Interpretation of haemolysin[4] was made after 48 hours' incubation at 37°C.

Crystal violet agar test The growth characteristics which appeared on the heart

infusion agar (Eiken) with crystal violet (Merck) in a final concentration of 1 : 200,000 were evaluated according to MEYER (1967).

Production of pigment The colony pigmentation was observed on a nutrient agar (Eiken) plate supplemented with 2 % skim milk (Difco) after incubation for 24 hours at 37°C and for 5 days at room temperature.

Bacteriophage typing The culture media used for propagation and titration of the phages and the typing of the staphylococcal isolates were those described by SHIMIZU (1977). The following 2 bacteriophage sets were used: 1) the basic set of 22 phages of the International series for typing human staphylococci supplied by M. OHASHI of the National Institute of Health, Tokyo; and 2) a set of 4 experimental phages[2)] derived from the staphylococci of canine origin, phage numbers 06, 40, 58, and 93, provided by A. SHIMIZU, Department of Animal Hygiene, the Faculty of Agriculture, Kobe University, Hyogo, Japan. The interpretation of the results of phage typing was performed according to BLAIR & WILLIAMS (1961). The staphylococcal isolates were tested with the phages in a 1× routine test dilution (RTD) and 100×RTD.

RESULTS

The exterior body sites and suppurative lesions of the household dogs (n=115) were examined for the presence of ES. Coagulase-positive staphylococci were found in a total of 99 (86.1 %) dogs, and ES were found in a total of 15 (13.0 %) dogs (tab. 1). The ES percentages detected in the dogs ranged from 11.1 to 17.9 %, with highest percents in August. Of the 9 laboratory dogs, coagulase-positive staphylococci were found monthly in all of the dogs, and ES were found twice in 2 dogs, in September and November, 1977, and in November, 1977 and July, 1978.

TABLE 1 *Occurrence of enterotoxigenic staphylococci in household dogs*

MONTHS OF 1977	NO. OF DOGS TESTED (a)	NO. OF DOGS FROM WHICH STAPHYLOCOCCI WERE ISOLATED		COLUMN (b) DIVIDED BY COLUMN (a)
		Coagulase-positive	Enterotoxigenic (b)	
July	39	33	5	12.8 %
August	28	24	5	17.9
September	22	18	3	13.6
October	18	17	2	11.1
November	8	7	0	0
Total (av)	115	99	15	(13.0)

The ES were isolated from the nasal cavity, external auditory canal, dorsal skin, anus and suppurative legions of household dogs in percentages ranging from 4.3 to

TABLE 2 *Frequency of enterotoxigenic staphylococci in exterior body sites of household dogs*

BODY SITES	NO. OF SAMPLES TESTED (a)	NO. OF SAMPLES FROM WHICH STAPHYLOCOCCI WERE ISOLATED		COLUMN (b) DIVIDED BY COLUMN (a)
		Coagulase-positive	Enterotoxigenic (b)	
Nasal cavity	115	65	7	6.1 %
External auditory canal	113	39	6	5.3
Dorsal skin	114	32	6	5.3
Anus	115	75	5	4.3
Exterior suppurative lesions	6	6	1	16.7
Total (av)	463	217	25	(5.4)

TABLE 3 *Biotypes of enterotoxigenic staphylococci isolated from household and laboratory dogs*

BIOCHEMICAL CHARACTERISTICS AND PHAGE SUSCEPTIBILITY	BIOTYPE*1			
	Household dogs			Laboratory dogs
	A (n=4)	B (n=1)	E (n=21)	E (n=6)
Fibrinolysin	4	0	0	0
Pigment	4	1	0	0
Coagulation of				
human plasma	4	1	0	0
bovine plasma	0	0	21	6
Haemolysin				
Alpha	2	1	0	0
Beta	0	1	21	6
Crystal violet test				
Orange	4	1	0	0
White	0	0	21	6
Susceptibility to phages*2				
International phages	3	1	0	0
Canine phages	0	0	13	6

*1 Biotype of the modified BAIRD-PARKER's classification described by HÁJEK & MARŠÁLEK (1973)

*2 100×routine test dilution

6.1 % (aver. 5.4 %) in all sites except the suppurative legions, where ES was found in 1 out of 6 legions (16.7 %) (tab. 2). The frequency of occurrence of enterotoxigenic isolates in the coagulase-positive staphylococci isolated from the household dogs was 26/254 (10.2 %) and from the laboratory dogs 6/374 (1.6 %).

Three biotypes, A, B and E, were found in the ES from the household dogs, and one biotype (E) was found in those from the laboratory dogs (tab. 3). Twenty-one toxigenic isolates from the household dogs belonged to biotype E (canine origin according to Baird-Parker's classification modified by HÁJEK & MARŠÁLEK), 4 isolates belonged to biotype A (human origin according to the classification), and 1 isolate belonged to biotype B (classified as swine or poultry origin). All 6 toxigenic isolates from the laboratory dogs belonged exclusively to biotype E.

The relation between the enterotoxin types and the biotypes of the enterotoxigenic isolates is shown in table 4. Of the 26 toxigenic isolates from the household dogs, 19 produced enterotoxin C, 6 produced enterotoxin A, and 1 produced enterotoxin D. None produced enterotoxins B or E. Of the 6 toxigenic isolates from the laboratory dogs, 3 produced enterotoxin C and 3 produced enterotoxins A+C. The biotype E isolates from the household dogs produced enterotoxin types A (3 isolates) and C (18 isolates). The biotype A isolates produced enterotoxin types A (3 isolates) and D (1

TABLE 4 *Relation between enterotoxin type and biotype*

DOGS	NO. OF ENTERO-TOXIGENIC ISOLATES	BIOTYPE:	A			B	E		
		ENTEROTOXIN TYPES:	A	C	D	C	A	C	A+C
Household	26		3	0	1	1	3	18	0
Laboratory	6		0	0	0	0	0	3	3

isolate). The biotype B isolate produced enterotoxin type C. The biotype E isolated from the laboratory dogs produced enterotoxins C (3 isolates) and A+C (3 isolates).

The relation between the isolated sites and the biotypes of the ES is shown in

TABLE 5 *Relation between the isolated sites of enterotoxigenic staphylococci and biotype*

DOGS	ISOLATED SITES:	NASAL CAVITY			EXTERNAL AUDITORY CANAL		DORSAL SKIN		ANUS	EXTERIOR SUPPURATIVE LESIONS
	BIOTYPE:	A	B	E	A	E	A	E	E	E
Household		1	1	5	1	5	1	5	5	1
Laboratory		0	0	3	0	2	0	1	0	0

table 5. In the household dogs, the isolates classified as biotpye E were detected in all of the sites, and all of the isolates belonging to biotype A were found in the nasal cavity, the external auditory canal, and in the dorsal skin. The isolate belonging to biotype B was found in the nasal cavity. The ES isolated from the suppurative legions belonged to biotype E and produced enterotoxin C. In the laboratory dogs, the isolates classified as biotype E were found in the nasal cavity, external auditory canal and dorsal skin.

The results of phage typing a total of 32 enterotoxigenic isolates are shown in table 6. Of the toxigenic isolates, a total of 17 of the biotype E isolates were lysed by phage 06 and 2 by phage 58 of the canine phage set, and 3 of the biotype A isolates were lysed by phage 53 (phage group III) of the human phage set. The biotype B isolate was sensitive to phages 52/52 A/80/6/47/53/75/42 D (phage group I/III/IV) of the human phage set. None of the biotype E isolates were sensitive to the human phages, and none of the biotype A and B isolates were lysed by the canine phages.

TABLE 6 *Biotypes and phage types of enterotoxigenic staphylococci isolated from dogs*

BIOTYPE	PHAGE TYPE				NO. OF ISOLATES		
	Canine phages		International series		Household dogs	Laboratory dogs	Total
	RTD*1	100×RTD	RTD	100×RTD			
E	06		—*2	—	11	4	17
	—	06	—	—	1	1	2
	58		—	—	1	1	2
	—	—	—	—	8	0	8
A	—	—	53		3	0	3
	—	—	—	—	1	0	1
B	—	—	—	52/52A/80/6/47/53/75/42D	1	0	1

*1 Routine test dilution
*2 Untypable

A small experiment was performed to demonstrate that the transmission of coagulase-positive staphylococci from the body surfaces of dogs to human hands is easily achieved by touching dogs. One of the authors wore sterile, vinyl medical gloves and held a laboratory dog while sampling its exterior body sites. The tests were repeated nine times for 9 different laboratory dogs, and upon examining the swabs obtained from the gloves, coagulase-positive staphylococci were isolated from all of the gloves.

DISCUSSION

The fact that 13.0 % of the household dogs examined possessed ES on their exterior body sites and suppurative legions is a significant finding which is of importance from the viewpoint of food hygiene. KATO et al. have studied the presence of ES in various sources. The frequency of carriers of enterotoxigenic strains among various hosts were, in the following decreasing order of frequency: 34.9 % in subclinical mastitis cows[8]; 22.0 % in food handlers of box lunches[11]; 5.8 % in stray dogs[7]; 3.8 % in chickens[15]; and 3.6 % in house rats[10]. ES were found more frequently in household dogs than in stray dogs, chickens, and house rats.

The present study shows that of the enterotoxigenic isolates, biotype E isolates (considered to be of canine origin by HÁJEK & MARŠÁLEK) produced enterotoxins A, C, and A+C, the biotype A isolates (considered to be of human origin) produced enterotoxins A and D, and the biotype B isolate (considered to be of swine or poultry origin) produced enterotoxin C. There have been only a few published accounts of enterotoxin production and biotyping of canine staphylococci. HÁJEK & MARŠÁLEK (1973) have found that 48 out of 58 strains isolated from dogs were classified as biotype E, but none produced enterotoxins. The remaining 10 strains belonging to biotype A produced mostly enterotoxins. KATO et al. (1978) attempted to isolate ES from the cecum and rectum of stray dogs in Sapporo. They found that biotype E isolates (canine origin) produced enterotoxin C exclusively, and pointed out the need for further investigations on the staphylococcal strains of canine origin, since they are potential sources of food poisoning.

The results of phage typing in the present study showed that the phage types of the biotype E isolates of the ES were 06 and 58, and that phage type 06 was predominant. The biotype A isolates were sensitive to phages of group III (phage type 53) and a mixed group of the human phage set. This result agrees with a previous finding of the ES isolated from stray dogs. In this study the biotype E isolates were lysed mainly by the phage 06 of the canine phage set, and the biotype A isolates were sensitive to phages of groups III and I of the human phage set[7].

During the present study, the authors have confirmed that coagulase-positive staphylococci present on the body surfaces of laboratory dogs were easily transmitted to the hands of a person who held and examined the dog. It is important to note the possibility that the ES present on the exterior body sites of household dogs held by humans may contaminate the food consumed by humans. Housewives, for example, who have had contact with household dogs may be crriers of ES, and thus, cause food to become contaminated. Since the ES isolated from the household dogs produced enterotoxins A, C, and D, which were the common enterotoxin types causing food poisoning in many countries[3,12,17,18], including Japan[16], the ES may be responsible for the development

of staphylococcal food poisoning in humans.

ACKNOWLEDGEMENTS

The authors wish to thank the staffs of the Veterinary Hospital and the Department of Surgery of the Faculty of Veterinary Medicine, Hokkaido University, Sapporo, for their assistance in obtaining samples from the dogs.

REFERENCES

1) BLAIR, J. E. & WILLIAMS, R. E. O. (1961): Phage typing of staphylococci *Bull. WHO*, **24**, 771–784
2) BLOUSE, L. & MEEKINS, W. E. (1968): Isolation and use of experimental phages for typing *Staphylococcus aureus* isolated from sentry dogs *Am. J. Vet. Res.*, **29**, 1817–1822
3) CASMAN, E. P., BENNETT, R. W., DORSEY, A. E. & ISSA, J. A. (1967): Identification of a fourth staphylococcal enterotoxin, enterotoxin D *J. Bacteriol.*, 94, 1875–1882
4) ELEK, S. D. & LEVY, E. (1950): Distribution of haemolysins in pathogenic and non-pathogenic staphylococci *J. Pathol. Bacteriol.*, **62**, 541–554
5) HÁJEK, V. & MARŠÁLEK, E. (1973): The occurrence of enterotoxigenic *Staphylococcus aureus* strains in hosts of different animal species *Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg., I. Orig.*, **A 223**, 63–68
6) KATO, E., KHAN, M., KUJOVICH, L. & BERGDOLL, M. S. (1966): Production of enterotoxin A *Appl. Microbiol.*, **14**, 966–972
7) KATO, E., KAJI, Y. & KANEKO, K. (1978): Enterotoxigenic staphylococci of canine origin *Am. J. Vet. Res.*, **39**, 1771–1773
8) KATO, E. & KUME, T. (1980): Enterotoxigenicity of bovine staphylococci isolated from California Mastitis Test-positive milk in Japan *Jpn. J. Vet. Res.*, **28**, 75–85
9) MEYER, W. (1967): Ueber die Brauchbarkeit des Kristallviolett-Testes zur Differenzierung von *Staphylococcus aureus*-Stammen *Z. Med. Mikrobiol. Immunol.*, **153**, 158–168
10) MORI, M., KATO, E. & HAMADA, S. (1977): Distribution of enterotoxigenic staphylococci in rats (*Rattus norvegicus*) and biological properties of isolates *Jpn. J. Bacteriol.*, **32**, 493–499 (in Japanese with English summary)
11) MORI, M., KATO, E. & HAMADA, S. (1977): Distribution of enterotoxigenic staphylococci in healthy food handlers and biological properties of isolates *Ibid.*, **32**. 501–508 (in Japanese with English summary)
12) NISKANEN, A. & KOIRANEN, L. (1977): Correlation of enterotoxin and thermonuclease production with some physiological and biochemical properties of staphylococcal strains isolated from different sources *J. Food Prot.*, **40**, 543–548
13) SATO, G., MIURA, S. & TERAKADO, N. (1972): Classification of chicken coagulase-positive staphylococci into four biological types and additional characteristics including coagulase-antigenic type *Jpn. J. Vet. Res.*, **20**, 91–110

14) SHIMIZU, A. (1977): Establishment of a new bacteriophage set for typing avian staphylococci *Am. J. Vet. Res.*, **38**, 1601–1605

15) SHIOZAWA, K., KATO, E. & SHIMIZU, A. (1980): Enterotoxigenicity of *Staphylococcus aureus* strains isolated from chickens *J. Food Prot.* (in press)

16) TERAYAMA, T. (1975): Coagulase type of *Staphylococcus aureus* isolates from food poisoning and their enterotoxin production *Mod. Media*, **21**, 436–440 (in Japanese)

17) UNTERMANN, F. & SINELL, H. J. (1970): Beitrag zum Vorkommen enterotoxinbildender Staphylokokken *Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg., I. Orig.*, **215**, 166–172

18) WIENEKE, A. A. (1974): Enterotoxin production by strains of *Staphylococcus aureus* isolated from foods and human beings *J. Hyg. (Camb.)*, **73**, 255–262