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CHARACTERIZATION OF *PASTEURELLA* FROM GINGIVAL SCRAPINGS OF DOGS AND CATS

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Abstract—Gingival scrapings of 62 dogs and cats were examined for the presence of *Pasteurella*. Isolation was performed in a medium supplemented with thiostrepton. Twenty-eight and 37 strains were obtained from 21 dogs and 26 cats, respectively, and classified in recently described species or subspecies of the genus *Pasteurella* (*P.*): *P. multocida* subspecies *multocida* and *septica*, *P. canis*, *P. dagmatis* and *P. stomatis*. Twenty-one strains were classified as atypical *P. stomatis* and one strain obtained from a cat remained unclassified. All strains were susceptible to the antibiotics studied. *P. multocida* and *P. stomatis* (including atypical strains) represented 65 and 30% of feline isolates, and 14 and 68% of canine isolates, respectively. Assuming that *P. multocida*, *P. canis* and *P. dagmatis* are potentially pathogenic for humans, and that *P. stomatis* has a low pathogenicity or non-pathogenic, 77 and 28% of examined cats and dogs harboured one or several pathogenic strains. This difference could explain the fact that *Pasteurella* infections in man are lower in dog bites rather than cat bites.

Key words: *Pasteurella* spp, characterization, frequency, dogs, cats, antibiotic sensitivity.

Résumé—La recherche de *Pasteurella* a été effectuée à partir de la cavité buccale des chiens et chats, sur un milieu additionné de thiostrepton. Vingt-huit et 37 souches ont été isolées respectivement de 21 et 26 prélèvements d'origines canine et féline. Ces souches ont été classées dans les espèces et sous-espèces du genre *Pasteurella* (P.), récemment décrites: *P. multocida* sous-espèces *multocida* et *septica*, *P. canis*, *P. dagmatis* et *P. stomatis*. Vingt-et-une souches atypiques sont proches de *P. stomatis* et une souche d'origine féline n'a pu être classée. Toutes les souches se révèlent sensibles aux antibiotiques étudiés. *P. multocida* et *P. stomatis* (y compris les souches atypiques) représentent respectivement 65 et 30% des isolements d'origine féline, 14 et 68% de ceux d'origine canine. En considérant que *P. multocida*, *P. canis* et *P. dagmatis* sont potentiellement pathogènes pour l'homme, mais que *P. stomatis* est peu ou non pathogène, 77% des chats et 28% des chiens hébergent une ou plusieurs espèces pathogènes dans leur cavité buccale: cette différence permet d'expliquer le fait que les infections pasteurelliques humaines sont moins fréquentes après morsure de chien que de chat.

Mots clefs: *Pasteurella*, caractérisation, fréquence, chiens, chats, sensibilité aux antibiotiques.

INTRODUCTION

Pasteurellosis of man is commonly associated with dog or cat bites and scratches [1–4]. Indeed, *Pasteurella* is a commensal bacteria currently isolated from the oral cavity of dogs and cats [5–10]. The carriage is reported in frequence varying between 22 and 81% [9, 10]

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rising up to 90% for cats [8]. This high carriage rate explains the frequent occurrence of *Pasteurella* infection in bite or scratch wounds.

Mutters *et al.* [11], by DNA–DNA hybridizations, proposed a new classification of the genus *Pasteurella* (*P.*). Bisgaard and Mutters [12] also detailed phenotypic characterization of some previously unclassified *Pasteurella* spp obtained from the oral cavity of dogs and cats. Among the species described, *P. multocida* is the most frequently found in bite or scratch wounds. Other species have also been isolated, particularly *P. canis* and *P. dagmatis* [1, 13, 14]. However, no report using this new classification gives data on the proportion of these different species in dogs and cats.

The purpose of the present study was: (a) to characterize *Pasteurella* found in gingival scrapings of dogs and cats, with reference to species previously reported as being associated with human infection resulting from these animal bites; (b) to estimate the healthy carrier's percentage; (c) to determine the pathogenicity of the different species isolated and (d) to compare their sensitivity to antibiotics with that of human *Pasteurella* isolates.

MATERIALS AND METHODS

Collection of specimens

Gingival scrapings were taken from 32 dogs and 30 cats of various ages, sexes and breeds, using sterile cotton-tipped swabs. All of the animals presented for vaccination at the Veterinary School of Nantes between January and April 1990 were considered healthy.

Culture and identification

Swabs were transported immediately to the laboratory and the specimens were rapidly streaked for isolation purposes on 5% sheep's blood agar (Columbia agar, Biomérieux) supplemented with thiostrepton (20 mg/l, Sigma). Plates were incubated at 37°C in ambient air for 48 h and suspect colonies were cultured for identification and conservation at −80°C. Identification of genus was done using conventional microbiological procedures, including susceptibility to the vibriostatic compound O/129, which was determined on Mueller–Hinton agar (Diagnostic Pasteur) supplemented with 0.1% yeast extract (Difco) [15].

The characterization of species and subspecies was performed using the API-20E and API-50CH system (Biomérieux). API-20E strips were used for indole, urea and ornithine decarboxylase tests, and API-50CH for carbohydrate fermentation tests. A bacterial suspension (corresponding to 4 Mc Farland standard) was inoculated to fill the cupules, in M63 medium as previously described [15]. Results were recorded after incubation period of 24–72 h at 37°C.

Mouse pathogenicity

Mouse pathogenicity of each isolate was determined. A 15–20 g Swiss mouse received intraperitoneally 0.3 ml of an 18 h broth culture. Death occurred within 72 h and *Pasteurella* was isolated from cardiac blood, which confirms its pathogenicity.

Antibiotic sensitivity

Strains were tested for susceptibility to antibiotics usually recommended for pasteurellosis treatment [1, 16, 17]: ampicillin, penicillin, cefotaxime, chloramphenicol, tetracycline and pefloxacine. Minimal inhibitory concentrations (MICs) were determined on

Mueller–Hinton agar supplemented with 5% sheep blood (haemolysed blood for pefloxacine).

RESULTS

Pasteurella were isolated scrapings in 21 out of 32 dogs and in 26 out of 30 cats (66 and 87%, respectively) from gingival scrapings. A single animal can be a carrier of several *Pasteurella* strains. As a result, 65 strains were characterized with 28 found in the 21 dogs and 37 strains found in the 26 cats.

Frequency of isolates and phenotypic characteristics obtained are shown in Tables 1 and 2. Twenty-eight strains corresponded to *P. multocida* subspecies *multocida* and *septica* (9 strains differed from typical species described, by acid production from maltose), 4 strains to *P. dagmatis* (formerly *P. pneumotropica* biotype Henriksen) and 2 strains to *P. canis* (formerly *P. multocida* biotype 6 or dog-type of Frederiksen). Nine strains are identified as *P. stomatis* and 21 strains which formed a homogeneous group considered as atypical *P. stomatis* by acid production from maltose. One strain (*Pasteurella* spp), positive in acid production from sorbitol and negative from mannitol remained unclassified. All strains were susceptible to the vibriostatic compound O/129.

In this experiment, differences in species distribution were observed (Table 3) between dogs and cats: 23 out of 30 (77%) examined cats harboured *P. multocida*, but only 4 out of 32 (13%) dogs. *P. stomatis* and atypical *P. stomatis* predominated in dogs: these strains represented 68% of canine isolates (Table 1) and were recovered from 17 out of 32 (53%) of the dogs examined (Table 3). *P. canis* was only found in the dogs.

A single animal can carry several species or subspecies, for example, *P. multocida* subspecies *multocida* and *septica* with atypical *P. stomatis* were obtained from gingival scraping of one dog and *P. dagmatis* with *P. stomatis* (or atypical *P. stomatis*) were recovered from one cat (Table 3).

Most of the *Pasteurella multocida* (23 out of 28) and 1 out of 2 *P. canis* strains were lethal for mice. On the other hand, 2 *P. stomatis* and 2 atypical *P. stomatis* strains were pathogenic and *P. dagmatis* showed no pathogenicity to mice (Table 2).

All strains were susceptible to ampicillin (MIC ≤ 0.5 mg/l), penicillin G (MIC ≤ 0.5 UI/l), cefotaxime (MIC ≤ 0.03 mg/l), chloramphenicol (MIC ≤ 1 mg/l), tetracycline (MIC ≤ 2 mg/l), and pefloxacine (MIC ≤ 0.125 mg/l). No difference in susceptibility was noted between *Pasteurella* species (Table 4).

Table 1. Isolation of *Pasteurella* species from dogs and cats

Species	Number of strains	
	Dogs	Cats
P. multocida		
subsp. *multocida*	2 (7)*	14 (38)
subsp. *septica*	2 (7)	10 (27)
P. stomatis (with atypical strains)	19 (68)	11 (30)
P. canis	2 (7)	0
P. dagmatis	3 (11)	1 (3)
Pasteurella spp	0	1 (3)
Total of isolates	28	37

*Number in parentheses indicate percentage values

Table 2. Characteristics of isolated *Pasteurella* species

	P. multocida subsp.										
	multocida ($n = 16$)		*septica* ($n = 12$)		*P. stomatis* ($n = 30$)		*P. canis* ($n = 2$)	*P. dagmatis* ($n = 4$)		*Pasteurella* spp ($n = 1$)
Characteristics	Dogs	Cats	Dogs	Cats	Dogs	Cats	Dogs	Dogs	Cats	Cat
Number of strains	2	14	2	10	19	11	2	3	1	1
Urease	−	−	−	−	−	−	−	+	−	−
Indole*	+	+	+	+	+	+	+	+	+	+
Ornithine-decarboxylase	+	+	+	−	−	+	+	−	+	+
Acid from =										
N-acetyl-glucosamine	+	+	+	+	+	+	+	+	+	+
Adonitol	−	−	−	−	−	−	−	−	−	−
Amygdalin	−	−	−	−	−	−	−	−	−	−
L-arabinose	−	2/14	−	−	−	−	−	−	−	−
Cellobiose	−	−	−	−	−	−	−	−	−	−
Ducitol	−	−	−	−	−	−	−	−	−	−
Esculin	−	−	−	−	−	−	−	−	−	−
Fructose	+	+	+	+	18/19	9/11	+	+	+	+
Galactose	+	+	+	+	15/19	7/11	1/2	+	+	+
Gluconate	1/2	8/14	+	+	12/19	8/11	+	+	+	+
D-glucose	+	+	+	+	+	+	+	+	+	+
Glycerol	1/2	1/14	−	2/10	−	−	−	−	−	−
Inositol	−	−	−	−	−	−	−	−	−	−
5-Cetogluconate	−	3/14	+	3/10	8/19	2/11	−	2/3	−	−
Lactose	−	−	−	−	−	−	−	−	+	+
D-lyxose	−	9/14	+	+	1/19	3/11	1/2	1/3	−	−
Maltose	1/2	2/14	1/2	5/10	13/19	8/11	−	+	+	+
D-mannitol	+	+	+	−	−	−	−	−	−	−
D-mannose	+	+	+	+	+	+	+	+	+	+
Melibiose	−	−	−	−	−	−	−	−	−	−
Raffinose	−	7/14	−	7/10	8/19	4/11	−	1/3	−	−
L-rhamnose	−	−	−	−	−	−	−	−	−	−
D-ribose	1/2	+	+	+	13/19	+	−	3/3	−	−
Salicine	−	−	−	−	−	−	−	−	−	−
D-sorbitol	+	+	−	−	−	−	−	−	−	+
Sucrose	+	+	+	+	17/19	10/11	1/2	2/3	+	−
Trehalose	1/2	4/14	+	9/10	12/19	8/11	1/2	1/3	+	−
D-xylose	1/2	11/14	+	8/10	12/19	−	−	1/3	−	−
Experimental infection	2/2	12/14	1/2	8/10	4/19	−	1/2	−	−	−

*Some strains give weak reaction; number/n = total number of positive strains; + = Positive reaction observed after 2 or 4 days; − = no reaction.

Table 3. Distribution of *Pasteurella* isolated from gingival scrapings of dogs and cats

	Pasteurella species
Cats ($n = 30$)	
4	No isolate
8	*P. multocida* subsp. *multocida*
6	*P. multocida* subsp. *septica*
3	*P. multocida* subsp. *multocida* + *P. stomatis*
1	*P. multocida* subsp. *multocida* + atypical *P. stomatis*
3	*P. multocida* subsp. *septica* + *P. stomatis*
1	*P. multocida* subsp. *multocida* + *P. multocida* subsp. *septica* + atypical *P. stomatis*
1	*P. multocida* subsp. *multocida* + *P. dagmatis*
1	Atypical *P. stomatis*
1	*P. stomatis*
1	*Pasteurella* spp (unclassified) + *P. stomatis*
Dogs ($n = 32$)	
11	No isolate
1	*P. multocida* subsp. *septica*
1	*P. multocida* subsp. *septica* + *P. stomatis*
1	*P. multocida* subsp. *multocida* + atypical *P. stomatis*
1	*P. multocida* subsp. *multocida* + *P. stomatis* + atypical *P. stomatis*
2	*P. canis*
1	*P. dagmatis*
1	*P. dagmatis* + *P. stomatis*
1	*P. dagmatis* + atypical *P. stomatis*
2	*P. stomatis*
9	Atypical *P. stomatis*
1	*P. stomatis* + atypical *P. stomatis*

n = number of animals.

DISCUSSION

The aerobic flora found in oral cavity of dogs and cats was varied [7, 10], and the number of different organisms (particularly Gram-positive bacteria) growing on blood agar is often high. Thiostrepton, a polypeptidic antibiotic, is inactive against *Pasteurella* species (MIC ≥ 128 mg/l) but inhibits the Gram-positive bacteria. Its incorporation into blood agar of primary plates reduces number of the bacteria and can greatly facilitate the detection and identification of the *Pasteurella* colonies, which can be differentiated to colonies of other Gram-negative bacteria. These results indicate that thiostrepton blood agar can be recommended as an isolation medium for *Pasteurella* from feline and canine origin and confirm the high frequence carriage in the oral cavity of dogs and particularly of cats. The frequency of *Pasteurella* isolates (66 and 87% in dogs and cats, respectively), was similar to results found in other data [5–9].

Table 4. Susceptibility to antibiotics among *Pasteurella* species

	MIC (mg/l)*					
Species	Ap	Pen	Ctx	Cm	Tc	Pef
P. multocida subsp. *multocida*	0.06–0.125	0.125–0.25	0.016–0.03	0.5–1	0.5–2	0.06–0.125
P. multocida subsp. *septica*	0.06–0.125	0.06–0.25	0.04–0.03	0.5–1	0.5–2	0.06–0.125
P. canis	0.06	0.125	0.016	0.5	0.5	0.125
P. dagmatis	0.06–0.125	0.25	0.08–0.016	0.5	0.5	0.125
P. stomatis (with atypical strains)	0.06–0.5	0.06–0.5	0.002–0.03	0.5–1	0.5–2	0.06–0.125
Pasteurella spp	0.03	0.06	0.04	0.5	0.5	0.125

Ap: ampicillin; Ctx: cefotaxime; Cm: chloramphenicol; Pef: pefloxacine; Pen: penicillin; Tc: tetracycline.
*Penicillin: UI/ml.

Several authors [12, 13, 18, 19] have underlined the heterogeneity of phenotypic characteristics from feline and canine *Pasteurella* strains. This variety is confirmed in our study by the identification of several species (*P. multocida* subspecies *multocida* and *septica*, *P. canis*, *P. dagmatis* and *P. stomatis*) among dog and cat strains. Our results are in accordance with Mutters *et al.*'s classification [11, 20] except few strains identified as *P. multocida* which are maltose-positive and some strains identified as *P. stomatis* which are raffinose-positive. However, we found several atypical *P. stomatis* strains that we have first considered as a maltose-positive biotype. It would appear that this homogeneous taxon corresponds to the provisionally called "Taxon 16" of Bisgaard and Mutters [12]. The atypical strain "19B", isolated from one cat is ODC+, maltose+, mannitol− and sorbitol+, and could be tentatively classified as mannitol-negative *P. multocida* subsp. *multocida*. But phenotypic separation between different species or subspecies within the *Pasteurella* genus depends on a limited number of characters [12, 20] and errors of identification are possible.

The mouse pathogenicity test as performed here is critical because it is based on the inoculation of a single mouse per isolate, even though the use of a five or ten mice group per isolate would have been preferable. Our results are, however, indicative. For Smith [19], most of the feline strains were highly pathogenic for mice, whereas canine strains (often with maltose-positive character) were generally of low pathogenicity or non-pathogenic. In Art's study [6], the proportion of pathogenic strains was also higher among the cat isolates.

Globally in our study, 8 out of 28 canine and 20 out of 37 feline isolates were lethal for mice, but in reality mouse pathogenicity varies with respect to the *Pasteurella* species. The majority of *P. multocida* (82%) were lethal for mice. Furthermore there was no relation between pathogenicity and the positive or negative maltose strains found. On the other hand, the majority of *P. stomatis* and atypical *P. stomatis* isolates were non-pathogenic and only 2 feline strains in each group were pathogenic. One of two *P. canis* was lethal for mice, but there were insufficient strains in order to make a conclusion. Finally, *P. dagmatis* is recognized to be non-lethal for mice after intraperitoneal experiments.

The frequency of different *Pasteurella* in dogs and cats is worth commenting on with regards to the proportion of *Pasteurella* associated with bites in man. In dogs, the predominating species are *P. stomatis* and atypical *P. stomatis* (68%). *P. multocida*, *P. canis* and *P. dagmatis* were recovered in similar proportions (7–14%). In cats, the predominating species is *P. multocida* (65%).

In humans, the *Pasteurella* species isolated from animal bites or scratches are usually *P. multocida* and yet in lower frequency *P. dagmatis* or *P. canis* [1, 14, 21]. In a recent study [22], among 265 strains isolated from animals wounds and collected at the *Pasteurella* National Centre of Institut Pasteur between 1985 and 1990, Escande relates 60% of *P. multocida* (41 and 20% subspecies *multocida* and *septica*, respectively), 24% of *P. canis*, 11% of *P. dagmatis*, but only 4% of *P. stomatis*. The proportions of *Pasteurella multocida* subsp. *multocida* and *septica* confirm previous reports showing *P. multocida* subspecies *septica* as less present than *multocida* subspecies [23]. These observations contrast with our results, indicating that *P. multocida* subspecies *multocida* and *septica* are distributed in equal proportion in dog and cat oral cavities.

Very little data is available on mixed infections in humans for several *Pasteurella* species following animal bites. Few cases of *P. multocida* and *P. dagmatis* infection resulting from

cat bites were reported [24, 25], and Escande [22] also found several double infections with *P. multocida* associated with *P. canis*, and *P. dagmatis* associated with *P. canis*. This occurrence can be explained by our study which shows the simultaneous presence of different *Pasteurella* species or subspecies in gingival scrapings of some dogs and cats.

Despite the fact that dogs are more frequently involved in bites than cats, the frequency of *Pasteurella* infection in man is lower after dog bites than following cat bites [2, 4, 26]. This difference could be explained by the fact that the cat, with its sharp canine teeth, can bring about more severe bite wounds. This may involve deeper punctures, affecting bones, joints and tendons, a situation which is more propitious to *Pasteurella* infection development [6]. The higher *Pasteurella* carriage in cats may be another explanation [8]. Smith [19] also indicated a possible difference in pathogenicity among *Pasteurella* isolated from dogs as compared to those from cats. Assuming that *P. stomatis* and atypical *P. stomatis* species are of low pathogenicity or non pathogenic, and that *P. multocida* (especially *P. multocida* subspecies *multocida*), *P. dagmatis* and *P. canis* are potentially pathogenic for human, our results are partially consistent with this hypothesis. Indeed, the pathogenic *Pasteurella* species carrier state in cats is significantly higher ($P \leq 0.05$) than that of dogs. In our study 23 out of 32 cats and only 9 out of 30 dogs harboured one or several potential pathogens.

According to Goldstein *et al.* [27], antibiotic susceptibility from feline and canine strains, with their various degrees of pathogenicity, may not be applicable to the clinical situation encountered in human infection. We think that the antibiotic sensitivity survey of these isolates could be an interesting indicator. In the present investigation, all the strains are uniformly sensitive to the tested antibiotics. No difference was found in susceptibility between the *Pasteurella* species of either feline or canine origin. Penicillin and tetracycline are classically regarded as the drugs of choice for pasteurellosis treatment in man [17, 26], but other active drugs, including ampicillin, parenteral cephalosporins, chloramphenicol [1] or more recently quinolones of third generation [16, 28, 29] should be prescribed. However, in previous reports, the antibiotic susceptibility (performed with the disk diffusion method) of animals isolates was the subject of controversial data. In Arnbjerb's study [5], 25 and 50% of canine and feline strains respectively were resistant to penicillin; in Art's study [6], 42 and 73% of canine and feline isolates were also resistant to penicillin, 6% of canine strains were resistant to ampicillin and 15% of feline strains were resistant to tetracycline. However, no penicillin resistance has been reported in *Pasteurella* strains isolated from human bite infections [26], and our MIC results are similar to those recently found in animal bite wound isolates [27, 30, 31]. Moreover, we failed to find penicillin resistance among strains isolated from pathological cases (bite wounds, abcess, respiratory or genital tract infections) in cats and dogs (unpubl. data). This situation also differs from those described in bovine and porcine isolates [32–34], for which the antibiotics use as preventive therapy in intensive production system has led to the emergence of multi-resistant *Pasteurella* strains [32, 34].

In conclusion, various *Pasteurella* species and subspecies, and also numerous atypical strains are currently isolated from the oral cavity of dogs and cats. Bacteriological examination of gingival scrapings of dogs and cats confirms this heterogeneity and shows that *Pasteurella* are not distributed in equivalent proportions in these animals. In cats, the predominating species is *P. multocida*, whereas *P. stomatis* (or atypical *P. stomatis*) prevails in dogs. On the other hand, in humans, *Pasteurella* species isolated from animal bite wounds are usually *P. multocida*, *P. canis*, *P. dagmatis* and exceptionally *P. stomatis*.

These observations should explain the classical observation that cat bite wounds are more often infected than dog bite wounds. All strains are susceptible to antibiotics possibly recommended for pasteurellosis treatment in humans, particularly penicillin G and tetracycline.

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