

SOME STUDIES ON *PASTEURELLA MULTOCIDA* IN MAN AND PET ANIMALS

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SUMMARY

Gingival scrapings of 75 dogs and cats (40 dogs- 35 cats) were examined for the presence of Pasteurella multocida. Pasteurella multocida isolated strains were represented by 33 canine isolates and 12 feline isolates, respectively. 50 Sputum samples from bronchial secretions of pet caretakers were admitted with a 48 hour history of headache, neck stiffness, central pleuritic chest pain relieved by sitting forward and made worse by lying flat, fever, rigors, and drowsiness, with history of contact with stray or Owens cats or dogs. 28 out of 50 cases were positive for Pasteurella multocida in (56%). Assuming that Pasteurella multocida is potentially pathogenic for pet caretakers, 60% of examined cats and dogs harboured pathogenic strain. Serotyping of the isolated strains from dogs, cats and man in the same locality was demonstrated in three serotypes of Pasteurella multocida (A1, A2 and A6) having the possibility of cross infection between dogs, cats and man. Antimicrobial sensitivity test proved that Pasteurella multocida isolated strains were sensitive to cephalothin (30 mg), Erythromycin (15mg), Streptomycin (10mg), Gentamycin (10mg) Amoxicillin (20mg), and medium resistant to Ampicillin (10mg).

INTRODUCTION

Pasteurellosis is a zoonosis caused by a Gram negative coccobacilli found in the nasopharynx and gastrointestinal tract of many animals. About 50%-90% of domestic cats and dogs carry pasteurella species in their saliva and nasal tract and the organism is frequently found in injuries from cat scratches (75%) and dog bites (50%). (Talan et al. 1999). Pasteurella multocida may cause local wound infections in man as a result of contact with animals

and serious systemic infection and septicemia had been reported including endocarditis, pericarditis, polyarthropathy, and death. (Jenkins, 1999). This case is believed to be the first reported instance of *Pasteurella* pericardial tamponade.

Pasteurella multocida is the principal agent of human *Pasteurellosis* and has been recovered from the sputum and bronchial secretions of humans suffering from bronchiectasis and other chronic pulmonary diseases and a part of the normal flora of the upper respiratory tract of dogs and cats (Pedro Acha and Boris Szyfres 1995). *Pasteurella multocida* is a species of small, bipolar staining coccobacillus residing, saprophytically, in the oropharynx of many domestic and wild animals. Infection or cellulitis caused by *Pasteurella multocida* most often occurs after contact with a dog or cat. The biochemical and serological characteristics of *Pasteurella multocida* strains isolated from 79 healthy dogs were type A (A1, A2, A5 and A6) (Dziva et al. 2000). The types (or serogroups) of *Pasteurella multocida* in dogs and cats were identified on the basis of differences in serotypes and the most present one in these species were type A. (Quinn et al. 2002)

The aim of current study was carried out to throw light on the following:

- The incidence of *Pasteurella multocida* isolates in cats and dogs.
- The incidence of *Pasteurella multocida* isolates in diseased pet caretakers.
- Serotyping of the isolated *Pasteurella multocida* to detect the zoonotic strains in both dogs and diseased dog caretakers.
- The Summarized results of antimicrobial sensitivity test of *Pasteurella multocida* isolated strains.

MATERIALS AND METHODS

Samples:

Samples used for bacteriological examination in this work had been obtained from 75 pet animals (40 dogs and 35 cats) and 50 pet caretakers. The samples were taken from gingival scraping of animals and sputum from bronchial secretions of pet caretakers. Gingival swab were taken from dogs and cats, which had been in contact with the pet caretakers according to Sheila Polakoff et al. (1967).

Media used :

1.2.1. Media used for cultivation and isolation:

1: Blood agar and chocolate blood agar media

They were used as enriched media for isolation, cultivation of delicate fastidious microorganisms. (Cruickshank et al. 1975).

2: DAS medium :

Crystal violet-cobalt agar was used as a selective medium for isolation of *Pasteurella* species due to ability to grow in the presence of 0.1% crystal violet (Cruickshank et al. 1975).

1.2.2. Media used for biochemical reaction :

The media used for biochemical reaction according to *Mackie and McCarty* (1996).

2: Methods:

2:1: Collection of samples :

The samples were collected by means of sterile cotton swabs and transport to laboratory as quickly as possible in sterile peptone water 1%

2:2: Bacteriological examination :

2:2:1: Primary isolation and purification:

The collected samples were inoculated directly into blood agar, chocolate blood agar and crystal violet cobalt agar plates that were incubated aerobically at 37° C for 48 hours. Suspected growing colonies onto the surface of above mentioned media were characterized on the basis of their colonial morphology and staining reactions (Cruickshank et al. 1975).

2:2:2: Identification of isolated strains:

Pure culture from each isolates were identified morphologically according to their stain reaction

Gram's stain: It was used to differentiate between the isolated organisms into the classical Gram positive and Gram-negative.

Leishman's stain: It was used to detect the *Pasteurella* bipolarity, microscopically the bacteria appear as tiny coccobacilli with bipolar staining. (Cruickshank et al. 1975).

Pure colonies were identified biochemically according to *Krieg and Holt* (1984)

*2:3: Serogrouping of the isolated *Pasteurella multocida* strains:*

Serotyping of the isolates for somatic antigen was performed by the gel diffusion precipitin test according to Heddleston et al. (1972). The 18-24 hour growth from a heavily seeded blood agar plate was suspended in 1 ml of 0.85% sodium chloride solution containing a 0.3% saturated solution for formaldehyde. The suspension of the cells was heated

hour. The cells were sedimented by centrifugation and the supernatant was used for the antigen in the gel diffusion precipitin test. The agar gel consisted of 0.9% Nobel agar (Difco), 8.5% sodium chloride and 0.01% thiomersal 5 ml of melted agar was placed on 25X75 mm microscope slide. Antisera were placed in outer wells and the antigens in center wells. Standard *Pasteurella multocida* typing antisera for serogroups A,B,D,E, and F were obtained from the Avrum Gudelsky Center for Veterinary Medicine, College Park, University of Maryland, U.S.A.

2:4:Antimicrobial sensitivity test:

In vitro antimicrobial testing for the isolated and identified bacterial isolates were performed according to Finegold and Martin, (1982).

The procedure was as follows:

A-Inoculation of plates:

1-Using a sterile loop, the top of four to five colonies were transferred to sterile test tube containing 5 ml of nutrient broth then incubated at 35°C for about 18 hours.

2-A sterile cotton swab was dipped into the standard bacterial suspension and streaked onto the dried surface of chocolate blood agar with an even distribution of the inoculum.

3-The plates were covered and remained for 5 minutes for absorption of the excess moisture.

4-By using a sterile pointed forceps, the antibiotic discs were placed on the inoculated plate and pressed firmly.

5-The discs distributed evening in a manner that they were away from the edge of the petri-dish by 15 mm and the distance between the centers of two discs not less than 24 mm.

B- Reading:

After incubation at 35°C for 24 hours, each plate was examined and the diameter of the complete inhibition zone (Clear Zone of inhibited growth around the discs) was measured by using reflected light and sliding caliper to the nearest millimeter. The diameters obtained zones of the bacterial isolates were interpreted by referring to the following table.

Zone interpretive standards of the National committee for Clinical Laboratory Standards (NCCLS) Finegold and Martin (1982).

Antimicrobial Agent	Disc potency	Inhibition	Inter - mediate	susceptible
Ampicillin	10 mg	20 or less	21-28	29 or more
Amoxicillin	20 mg	19 or less	--	20 or more
Cephalothin	30 mg	14 or less	15-17	18 or more
Erythromycin	15 mg	13 or less	14-17	18 or more
Gentamycin	10 mg	12 or less	13-14	15 or more
Streptomycin	10 mg	14 or less	15-18	19 or more

Antimicrobial agents were reconstituted according to the manufacturer instructions

RESULTS AND DISCUSSION

Microscopical character of *Pasteurella multocida*. By using bipolar staining, similar to closed safety pins.

Identification of *Pasteurella* isolates according to MacFaddin (1980) showed that :Glucose metabolism (oxidative-fermentative); production of indole, urease, and catalase-positive, oxidase-positive and indole-positive gram-negative coccobacillus grow on sheep blood , chocolate blood agar and Crystal violet- cobalt agar plates. From Table (1) the isolation and identification among cats and dogs revealed that out of 40 examined dogs, 33 gave positive results, with infectious rate 82.5%. But in case of cats 12 out of 35 examined gave positive results, with infection rate 34.2%. Out of 75 examined pets animal 45 were positive with infection rate 60% this indicates that the infection rate was higher in dogs than in cats. This finding agreed with that reported by Biberstein et.al. (1991). Out of 50 examined persons 28 were positive with infection rate 56%. This finding agreed with that reported by Bisgaard and Falsen. (1986). Humans have very frequent and close contacts with pets, an often forgotten source of zoonoses. In the particular case of *Pasteurella* infections, animal bites and scratches are the major sources of infection for humans Holmes et al.(1999). Others have suggested inhalation or licking and oral transmission as a possible infection route for those patients without injury Holst et al (1992). Finally, the present study stress on the danger of casual contact between humans and pets. The same observation was reported by Genne et al.(1996). From table (2) it is evident that out of 33 *Pasteurella multocida* isolates from dogs,17 were typed as (A1,A2,A5 and A6), while 16 untyped and out of 12 *Pasteurella multocida* isolates from cats, 7 were typed as (A1,A2,A5 and A6),

while 5 untyped . On the other hand, out of 28 human isolates in the same locality of dogs, and cats 22 were typed as (A1,A2 and A6), 6 untyped. The possibility of cross infection between dogs, cats and man were demonstrated by three serotypes A1, A2 and A6, similar results were recorded by Folder et al.(1999). Table (3) and Fig. (2) explain that the antimicrobial sensitivity test proved that *Pasteurella multocida* isolated strains were sensitive to cephalothin (30 mg), Erythromycin (15mg), Streptomycin (10mg), Gentamycin (10mg) Amoxicillin (20mg), and medium resistant to Ampicillin (10mg).

CONCLUSION

The interesting aspect about this illness is that it was caused by a cat bite. This emphasizes the importance of considering this unusual infection in all animal inflicted injuries. An inquiry revealed that the human had been in close contact with the dogs and possibly a cat through a misguided attempt by the parents to promote bonding between the human and the family's pets. This suggested a probable animal source of infection This case stresses the necessity of informing susceptible hosts at risk of contracting zoonotic agents about some basic hygiene rules when keeping pets. The most ecological evidence warns that better control of antibiotics on an international scale is the key factor needed to reduce the emergence of antibiotic-resistant organisms, including their maintenance in carriers. It may be necessary to avoid such practices as prophylactic and broad-spectrum therapy, therapy without sensitivity testing, and dissemination of residual antibiotics into the environment of man and animals

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Table (1) The percentage of *Pasteurella multocida* isolated from cats , dogs and pet caretakers:

Species	No. of samples	No. of positive cases	% of positive cases
Dogs	40	33	82.5%
Cats	35	12	34.2 %
Pet caretakers	50	28	56%

% Was calculated according to the No. of examined cases in each species

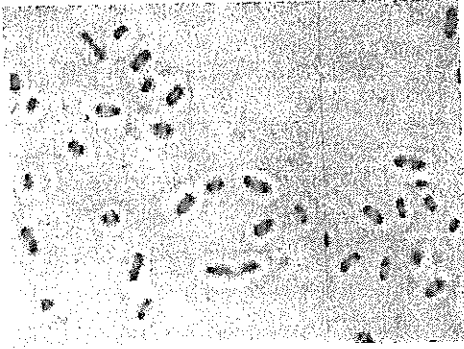
Table (2) Serotyping of isolated strains from Cat, dog and their care- takers:

Source of samples	Isolates	Typeable strains	Untypeable strains	Serotypes of typeable strains	Possibility of cross infection between man and dogs & cats
Man	28	22	6	A1,A2 ,A6	A1, A2 and A6
Dogs	33	17	16	A1A2,A5A6	
Cats	12	7	5	A1,A2,A5A6	

Table (3) The Summarized results of antimicrobial sensitivity test of *Pasteurella multocida*:

Antimicrobial agents	Disc potency	Inhibited zone	Results
Ampicillin	10mg	25	<u>M</u>
Amoxicillin	20 mg	25	<u>S</u>
Cephalothin	30mg	20	<u>S</u>
Erythromycin	15mg	25	<u>S</u>
Gentamycin	10 mg	22	<u>S</u>
Streptomycin	10 mg	22	<u>S</u>

S: susceptible M: Intermediate



Pasteurella multocida.
for typical bipolar staining

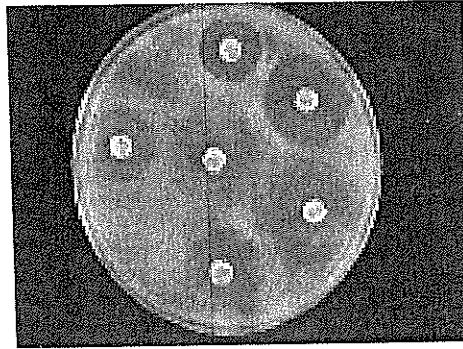


Fig.(2) Antibiotic susceptibility test
Pasteurella multocida