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Research Article

Bacteriological Analysis of a Lethal Outbreak of *Pasteurella canis* in Spotted Deer (*Axis Axis*) in a Zoological Park in Bareilly, India

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Abstract

The microbiological investigation of deaths of six spotted deer (*Axis axis*) in three days in a zoological park in Bareilly, India revealed *Pasteurella canis* of resistotype B (resistant to cotrimoxazole, enrofloxacin, meropenem, cefoxitin and erythromycin) as the cause of the disease as it was isolated from heart blood of all the six dead deer. However, from the same herd of the deer four different resistotypes (A, B, C, D) of *P. canis* were detected, but type A, C and D were detected from 5, 2 and 1 deer, respectively. Of the 20 isolates of *P. canis* from deer 12 had multiple drug-resistance (MDR) and 11 were resistant to meropenem also. The six isolates from three of the ten stray dogs in the vicinity of the park harboured *P. canis* in their oral cavity but all isolates belonged to resistotype E (resistant to erythromycin only) indicating that the lethal strain of *P. canis* isolated from spotted deer had a different origin than stray dogs. Enrofloxacin therapy in a sick deer failed to protect that but the incorporation of doxycycline (2 mg/ mL) in water was successful in preventing the deaths and illness in deer of the zoological park.

Keywords: Pasteurellosis; MDR; Blackbuck; Hog-Deer; Carbapenem-Resistance; Pneumoeteritis

Introduction

Pasteurellosis is an endemic disease in India caused by *Pasteurella* species, primarily *Pasteurella multocida* and *Pasteurella canis* [1]. They account for significant health and economic losses, especially in the livestock sector and poultry farming [2]. Zoonotic transmission to humans generally occurs through animal bites or licking or direct contact with nasal secretions [3]. As a zoonotic infection *Pasteurella canis* is often associated with soft tissue, bone, joint, and wound infections in humans acquired on bites and scratches by dogs and cats [4,5]. *Pasteurella canis* have been reported in 2.8 to 20% of the specimen of dog oral cavity, including both healthy dogs and dogs with periodontal diseases [6,7] and also from the respiratory tract of horses [8]. Though a few *P. canis* isolates have been reported to secrete toxins, the complete nature of the toxin and its role in pathogenicity is not lucid [9].

Most of the animals generally harbours *Pasturella canis* without showing any clinical signs but it in a few it may lead to a range of pathological symptoms from asymptomatic or mild chronic upper respiratory inflammation to acute or fatal pneumonic and/or disseminated disease leading to significant morbidity [10-12]. It has also been associated with superficial infections such as pyoderma, cutaneous abscesses [1, 13, 14], penetrating infection of eye [15], external otitis, rhinitis, vertebral osteomyelitis, meningomyelitis, bronchopneumonia, tracheitis, paranasal sinus inflammation, toxicosis [16-18] and endocarditis [19]. In cattle, it causes mild to severe pneumonia and different ailments in sheep, cats, rabbits, and deer [20].

Pasteurella canis are often penicillin-sensitive and multiple drug resistance is rarely reported [21,22]. Antimicrobial therapy results reported successful recovery after amoxicillin/clavulanic acid, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, doxycycline, fluoroquinolones, third and fourth generation cephalosporins and carbapenems [21] while macrolides, first-generation cephalosporins, and aminoglycosides are shown as unsatisfactory options [22]. There are several reports of pasteurellosis in deer due to *P. multocida* [23-26], but *P. canis* is rarely reported to cause fatal disease in deer. We report the death of six adult deer due to *P. canis* infection in a small herd of 30 adults raised in an enclosed and protected area of woods and fed with cultivated green fodder.

Case History and Animals

In a small zoological park in Bareilly, India spread over five acres of a protected area bounded with a brick wall on two sides and by steel railing and mesh (9 ft high) on other two sides had 30 spotted deer (*Axis axis*), 14 blackbucks (*Antilope cervicapra*) and 11 hog deer (*Hyelaphus porcinus*). An adult spotted deer having barbed wire injury was found dead on 28th Sept. 2019. Besides, one dear was sick with rectal temperature 102.5, keeping itself isolated and lagging due to malaise. It was treated with 50mg of flunixin meglumine, 100mg enrofloxacin and 8mg dexamethasone intramuscular injection, but it was found dead next morning. Rest of the herd was dewormed using triclabendazole 120mg and levamisole 75mg per 10 kg body weight. Next day (on 29 September) three more, including one treated, and on 3rd day (30 September) two more spotted deer were found dead in the morning. None of the five spotted deer died on 29th and 30th September 2019 had any lesion indicating injury. Heart blood samples from all the cases were submitted for culture to epidemiology

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Table 1: Antimicrobial resistance pattern of *Pasteurella canis* isolates from spotted deer (*Axis axis*) and stray dogs in the vicinity of zoological park in Bareilly, India.

Resistotypes	Resistant to	No. of isolates	Sample numbers
A	Cotrimoxazole and erythromycin	8	119, 122, 124, 125, 126
B	Cotrimoxazole, enrofloxacin, meropenem, cefoxitin, erythromycin	9	119, 122, 123, 124, 125, 126
C	Ampicillin, penicillin, cotrimoxazole, enrofloxacin, tetracycline, gentamicin, meropenem, imipenem, amoxicillin, azithromycin, erythromycin, cefotaxime, ceftriaxone	2	123, 124
D	Ampicillin, penicillin, cotrimoxazole, ceftazidime, tetracycline	1	124
E	Erythromycin	6	138, 139, 140 (all apparently healthy dogs)

laboratory for bacteriological examination and the Centre for Animal Disease Research and Diagnosis for viral agents. On 30 of September 2019 doxycycline (2 mg/ mL) was given in drinking water to the herd for three days and no more deaths were observed further. In postmortem reports, pneumoenteritis was recorded as a cause of the death without any more details and no Peste des Petits Ruminants **(PPR)** virus could be detected.

Follow-up Sampling

On suspecting dog bite as a source of *P. canis*, in the first week of October 2010 oral swabs of 10 stray dogs in the vicinity of the park were collected by sedating dogs through feeding them coconut cookies (one piece for a dog) containing 20 mg of diazepam each. All swabs were brought to laboratory within half h of collection for processing for the isolation of *P. canis*.

Bacteriological Examination

Each of the blood samples was streaked in triplicate on blood agar plates [blood agar base, (Difco, USA) with 5% sterile sheep blood]. One plate incubated aerobically at 37°C for 24 h, one plate at 37°C for 48 h in a microaerobic atmosphere with 5% $C0_2$, and the third plate was incubated anaerobically in jars with 90% H2 + 10% $C0_2$ and palladium catalyst at 37°C for 48 h. Three to five isolated colonies were picked up from each plate for further characterization based on growth, staining and biological characteristics [27] following criteria laid down in Bergey's Manual of Determinative Bacteriology [28]. After confirmation of the isolates, all were submitted for further confirmation to *Pasteurella* Reference Laboratory, ICAR-IVRI, Izatnagar. All different resistotypes of *P. canis* were deposited in the repository of Veterinary Type Culture Collection (VTCC), ICAR-IVRI, Izatnagar for future reference. All the cultures were maintained on blood agar slants until the end of the study.

All oral swabs of stray dogs were processed in the same way as the blood samples from deer but inoculated blood agar plates were incubated at 37oC for 24 h aerobically, only. The typical non-haemolytic, small transparent colonies showing oxidase-positive reaction using disc method [27] were further characterized for characteristic *P. canis* (producing oxidase, catalase and indole, fermenting glucose and sucrose but negative in methyl red and Voges Proskauer tests, not fermenting lactose, sorbitol and mannitol, not producing H^2S, urease, gelatinase, gas in glucose and lysine decarboxylase negative, and not growing on MacConkey's agar) as for those isolated from spotted deer.

Antimicrobial Sensitivity assay: All the isolates from spotted deer and dogs were tested for their sensitivity to amoxicillin (30µg), amoxicillin (30µg) + clavulanic acid (10µg), ampicillin (10µg), azithromycin (15µg), aztreonam (30µg), cefotaxime (10µg), cefoxitin (10µg), ceftazidime (30µg), ceftriaxone (10µg), chloramphenicol (25µg), colistin (10µg), cotrimoxazole (25µg), doxycycline (30µg), enrofloxacin (10µg), erythromycin (15µg), gentamicin (30µg), imipenem (10µg), meropenem (10µg), nitrofurantoin (300µg), penicillin G (10 IU), piperacillin (100µg), piperacillin (100µg) + tazobactam (10µg), tetracycline (30µg) and tigecycline (15µg) discs (Difco, USA) as per CLSI guidelines [29] on Mueller Hinton agar (MHA, Difco) supplemented with 5% bovine calf serum (Hi-Media, Mumbai) plates.

Results and Discussion

From blood samples of all the six spotted deer *P. canis* was isolated in pure culture. Of 20 isolates tested for their sensitivity to 24 antibiotics, they could be classified into four resistotypes, type A to type D (Table 1). Type B resistotype (resistant cotrimoxazole, enrofloxacin, meropenem, cefoxitin, erythromycin) was detected in blood samples of all the six spotted deer while other resistotypes were not detected from all the animals. The observations indicated that resistotype type B might be associated with the mortality in spotted deer. Type A detected in blood of five deer might be not important as it was sensitive to enrofloxacin but the deer treated with enrofloxacin also died i.e., antibiotic was probably not effective as the infection might be due to enrofloxacin resistant type B isolates. Similarly, type C and type D isolates may not be the cause of the mortality in spotted deer as they were detected only from two and one deer, respectively. Considering the sensitivity of all the *P. canis* isolates to doxycycline it was chosen for the therapy and the stoppage of death episode after inclusion of doxycycline in water indicated that *P. canis* might be the cause of lethality in spotted deer.

Of the 10 dogs in the vicinity of the zoological park, three were carrying *P. canis* in their oral cavity. However, all the six isolates from dogs belonged to a separate resistotype, sensitive to all antibiotics except erythromycin. It indicated that the disease-causing *P. canis* might not be from the dogs and probably evolved either in the deer park or might have come through birds perching in the park or other sources.

Park authorities not allowed sampling the remaining deer due to ethical and technical limitations for further investigation of the distribution of the pathogen in other spotted deer, hog deer and blackbucks.

In study, 17 isolates of *P. canis* from deer and six from dogs were sensitive to penicillin and ampicillin. The observations are in concurrence to earlier reports on *P. canis* sensitivity to penicillins [21, 22]. In study, 25 of 26 isolates were resistant to erythromycin and two to azithromycin indicating inefficacy of macrolides against

P. canis as reported earlier [22]. Though earlier studies indicated rarity of multiple drug resistance (MDR) among *P. canis* isolates [21, 22], in the present study >46% (12 of 26) isolates had MDR. The sensitivity of 24 of the 26 isolates to gentamicin in also in contrast to an earlier report indicating inefficacy of aminoglycosides against *P. canis* isolates [22]. An important observation was the resistance of 11 *P. canis* isolates from spotted deer to meropenem and two to meropenem and imipenem both indicated emergence of high-level drug resistance among *P. canis* isolates and necessitate further studies to understand the phenomenon of the unusual resistance among *P. canis* isolates. Resistance to carbapenems but sensitivity to penicillins observed in nine isolates of *P. canis* in the study needs further investigation as it is a rare trait among Gram-negative bacteria but reported in some of the streptococci due to some variation in penicillin-binding proteins [30]. The investigation indicated that *P. canis* can cause lethal infections and emergence of MDR in *P. canis* might be alarming and suggested use of antimicrobial sensitivity for instituting a successful antimicrobial therapy for *P. canis* infections.

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References

1. Talan DA, Citron DM, Abrahamian FM, Moran GJ, Goldstein EJ. Bacteriologic Analysis of Infected Dog and Cat Bites. Emergency Medicine Animal Bite Infection Study Group. N Engl J Med. 1999; 340: 85-92.
2. Souza MJ. Onehealth: Zoonoses in the Exotic Animal Practice. Vet Clin North Am Exot Anim Pract. 2011; 14: 421-426.
3. Wilson BA, Ho M. *Pasteurella multocida*: from Zoonosis to Cellular Microbiology. Clin Microbiol Rev. 2013; 26: 631-655.
4. Krol J, Bania J, Florek M, Pliszczak-Król A, Staroniewicz Z. Polymerase Chain reaction-based Identification of Clinically Relevant Pasteurellaceae Isolated from Cats and Dogs in Poland. J Vet Diagn Invest. 2011; 23: 532-537.
5. Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S. Bite-Related and Septic Syndromes Caused by Cats and Dogs. The Lancet Infect Dis. 2009; 9: 439-444.
6. Meyers B, Schoeman JP, Goddard A, Picard J. The Bacteriology and Antimicrobial Susceptibility of Infected and Non-Infected Dog Bite Wounds: Fifty Cases. Vet Microbiol. 2008; 127: 360-368.
7. Riggio MP, Lennon A, Taylor DJ, Bennett D. Molecular Identification of Bacteria Associated with Canine Periodontal Disease. Vet Microbiol. 2011; 150: 394-400.
8. Biberstein EL, Jang SS, Kass PH, Hirsh DC. Distribution of Indole-Producing Urease-Negative Pasteurellas in Animals. J Vet Diagn Invest. 1991; 3: 319-323.
9. Holst E, Rollof J, Larsson L, Nielsen J.P. Characterization and Distribution of *Pasteurella* Species Recovered from Infected Humans. J Clin Microbiol. 1992; 30: 2984-2987.
10. Harper M, Boyce JD, Adler B. *Pasteurella multocida* Pathogenesis: 125 Years After Pasteur. FEMS Microbiol Lett. 2006; 265: 1-10.
11. Hill WA, Brown JP. Zoonoses of Rabbits and Rodents. Vet Clin North Am Exot Anim Pract. 2011; 14: 519-531.
12. Souza MJ. Bacterial and Parasitic Zoonoses of Exotic Pets. Vet Clin North Am Exot Anim Pract. 2009; 12: 401-415.
13. Hara H, Ochiai T, Morishima T, Arashima Y, Kumasaka K, Kawano KY. *Pasteurella canis* Osteomyelitis and Cutaneous Abscess after A Domestic Dog Bite. J Am Acad Dermatol. 2002; 46: S151-152.
14. Ramiro GV, Gregorio AA, Luis GVJ, Gerardo DGL, Gerardo DCL. *Pasteurella Canis* as a Cause of Septic Arthritis and Soft Tissue Infection after Sheep Bite: A Case Report. Global J Med Clin Case Reports. 2016; 3: 12-14.
15. Rashid NK, Zam Z, MdNoor SS, Siti-Raihan I, Azhany Y. *Pasteurella canis* Isolation following Penetrating Eye Injury: A Case Report. Case Reports in Ophthalmological Med. 2012; 1-3.
16. Yefet E, Abozaid S, Nasser W, Peretz A, Zarfin Y. Unusual infection--*Pasteurella canis* Bacteremia in a Child After Exposure to Rabbit Secretions. Harefuah. 2011; 150: 13-15.
17. Csébi P, Jakab C, Jánosi K, Sellyei B, Ipolyi T, Szabó Z, et al,. Vertebral Osteomyelitis and Meningomyelitis Caused by *Pasteurella canis* in a Dog--Clinicopathological Case Report. Acta Veterinaria Hungarica. 2010;58: 413-421.
18. Hazelton BJ, Axt MW, Jones CA. *Pasteurella canis* Osteoarticular infections in Childhood: Review Oof Bone and Joint Infections Due to *Pasteurella* Species Over 10 Years at A Tertiary Pediatric Hospital and in The Literature. J Pediatric Orthopaedics. 2013; 33: 34-38.
19. Kern ZT, Swartley OM, Neupane P, Balakrishnan N, Breitschwerdt EB. *Pasteurella canis* Infective Endocarditis in A Dog. Vet Microbiol. 2018; 229: 14-19.
20. Mutters R., Ihm P., Pohl S., Frederiksen W, Mannheim W. Reclassification of the Genus *Pasteurella* Trevisan 1887 on the Basis of Deoxyribonucleic Acid Homology, with Proposals for the New Species *Pasteurella dagmatis, Pasteurella canis, Pasteurella stomatis, Pasteurella anatis, and Pasteurella langaa*. Int J Syst Evol Microbiol. 1985; 35: 309-322.
21. Kim B, Pai H, Lee KH, Lee Y. Identification of *Pasteurella canis* in a Soft Tissue Infection Caused by A Dog Bite: The First Report in Korea. Ann Lab Med. 2016; 36: 617-619.
22. Bhat S, Acharya P, Biranthabail D, Rangnekar A, Shiragavi S. A Case of Lower Respiratory Tract Infection with Canine-Associated *Pasterurella canis* in A Patient with Chronic Obstructive Pulmonary Disease. J Clin Diagn Res. 2015; 9: 3-4.
23. Carrigan MJ, Dawkins HJ, Cockram FA, Hansen AT. *Pasteurella multocida* Septicaemia in Fallow Deer (Dama dama). Aust Vet J. 1991; 68: 201-203.
24. Rajgopal R, Indu K, Nair GK. An Unusual Form of Pasteurellosis In Spotted Deer (Axis axis). Zoos' Print J. 2010; 25: 36-37.
25. Ravishanakar C, Antony PX, Biju KG. Pasteurellosis in a Herd of Spotted Deer (Axis axis). Zoos' Print J 2004; 19: 1493.
26. Eriksen L, Aalbæk B, Leifsson PS, Basse A, Christiansen T, Eriksen E et al. Hemorrhagic Septicemia in Fallow Deer (Dama dama) Caused by *Pasteurella multocida multocida*. J Zoo Wildlife Med. 1999; 30: 285-292.
27. Singh BR. Labtop for Microbiology Laboratory. Lambert Academic Publishing: Germany. 2009.
28. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams & Wilkins, Baltimore, MD. 1994.
29. M45. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 3rd edn. Clinical and Laboratory Standards Institute, Wayne, USA. 2015.
30. Chen Y, Hsieh Y, Gong Y, Liao W, Li S, Chang I et al,. Genomic Insight into the Spread of Meropenem-Resistant *Streptococcus pneumoniae* Spain23F-ST81, Taiwan. Emerg Infect Dis. 2020; 26: 711-720.