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Case report

Pasteurella canis soft tissue infection after a cat bite – A case report

Hristina Y. Hitkova [a,*], Preslava M. Hristova [a], Raina T. Gergova [b], Alexandra S. Alexandrova [b]

[a] Department of Microbiology and Virology, Faculty of Pharmacy, Medical University – Pleven, Pleven, Bulgaria
[b] Department of Medical Microbiology, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria

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ABSTRACT

Pasteurella canis is a Gram-negative facultative anaerobic bacterium that is a typical commensal of the oral cavity and enteric tract of domestic animals. Human infections caused by this species are most often associated with dog bites and rarely with other animal bites. In this report, we present a case of a 34-year-old man who developed *P. canis* soft tissue infection of the left hand after a cat bite. The patient was successfully treated by a surgical intervention and antibiotics. The species identification of the isolate was performed by a conventional automatic system and nucleic acid-based methods. The nucleic acid-based methods provide a powerful alternative to the conventional microbiological diagnostic of these bacteria.

Introduction

Pasteurella canis is a member of genus *Pasteurella* together with other species such as *Pasteurella multocida* (subspp. *multocida*, *septica,* and *gallicida*), *Pasteurella dagmatis* and *Pasteurella stomatis*. In the past, *P. canis* was considered as a biotype 6 or "dog type" of *P. multocida*, but in 1985 it was reclassified as a definitive species based on DNA homology [1]. *Pasteurella* spp. are Gram-negative facultative anaerobic rods or coccobacilli which are a part of the normal microbial flora of the oropharynx and the gastrointestinal tract of the domestic animals. The majority of human infections caused by *Pasteurella* spp. are associated with dog and cat bites, occasionally with bites of other animals or contacts with animals without bites and scratches, as well as diseases unrelated to animal exposure [2,3]. The most common species with clinical importance is *P. multocida,* but infections due to other species has also been reported [2,4].

P. canis is a rare pathogen that is related to dogs and occasionally to cats. It can cause a variety of infections – skin and soft tissue infections, arthritis, osteomyelitis, respiratory tract infections, eye infections, bacteremia, and others [5–11].

Case presentation

34-year-old man with a soft tissue infection of the left hand was admitted to a surgical ward at the University Hospital-Pleven, Bulgaria. Two days earlier, he was bitten by a stray cat on his left index finger. In the emergency room, the wound was dressed and antibiotic clindamycin was applied. On the next day, the wound started to get worse and a fever appeared.

Upon admission, the patient was in a good general condition without underlying diseases. The vital parameters were as follows: blood pressure – 125/90 mmHg, pulse – 80/min, respiration – 20/min, temperature – 37.6 °C. The laboratory tests were at normal levels (WBC – 11.0 × 10^9/L with 82.6 % neutrophils, RBC – 5.33 × 10^9/L, HGB – 162.0 g/L) with an exception of mildly elevated C-reactive protein (CRP) – 89.78 mg/L.

The erythema and edema involved the back of the palm and the dorsal surface of the left index finger of the patient. Four punctiform lesions from an animal bite were observed on the dorsal surface of the first phalanx of this finger. The local lymphangitis was not visualized, and enlarged lymph nodes were not detected in the cubital fossa or the left axilla. X-ray was performed, but data on traumatic bone changes were missing.

On the third day of hospitalization, the patient underwent a surgical intervention, during which a purulent exudate was discharged. A specimen from the exudate was collected for microbiological examination. The lavage was performed and three drains were placed. In the beginning, antimicrobial treatment included the combination of clindamycin, amikacin and metronidazole. Subsequently, on the basis of the susceptibility results, the antibiotic therapy was changed and levofloxacin was applied. On hospital day 11, the patient recovered and was discharged from the surgical ward.

* Correspondence to: Department of Microbiology and Virology, Faculty of Pharmacy, Medical University – Pleven, 1 St. Kliment Ohridski Str., 5800 Pleven, Bulgaria.
E-mail address: hitkova@abv.bg (H.Y. Hitkova).

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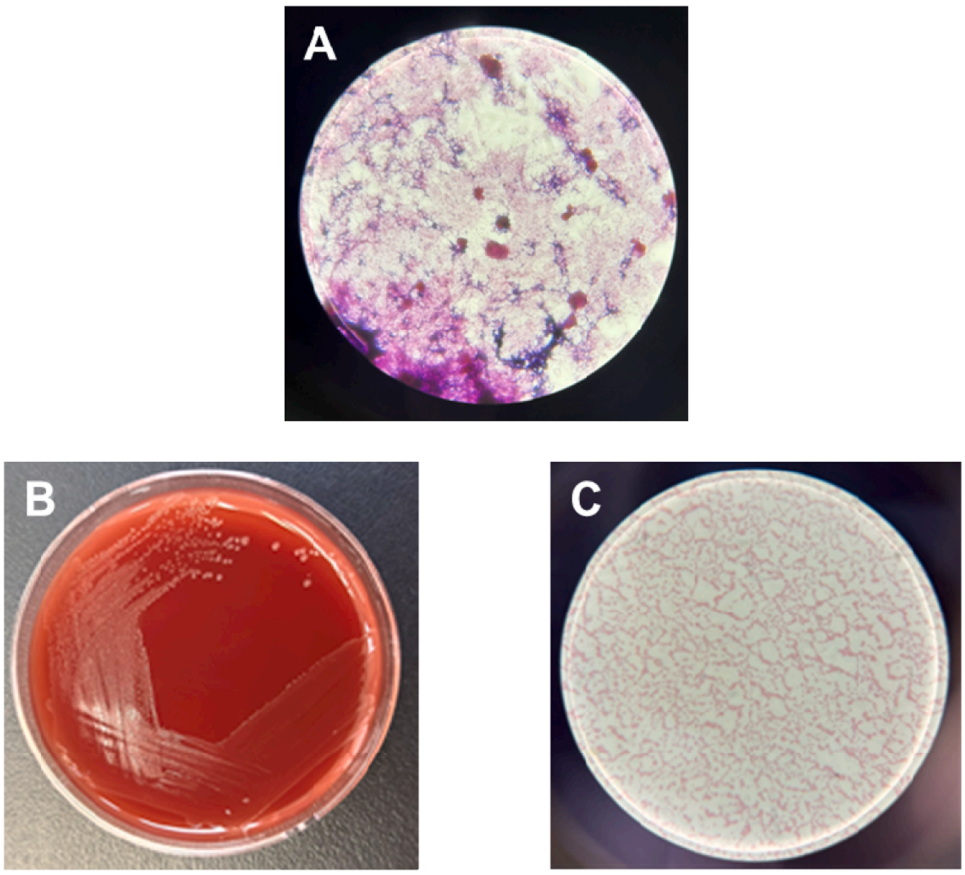

Fig. 1. Microscopy and cultures of *P. canis* isolate **(A)** Gram-stained smear from the purulent exudate (× 1000)**;(B)** Colonies of *P. canis* on the BAP; **(C)** Gram-stained smear from the colonies (× 1000).

Performed microbiological testing included a Gram-stained smear and cultures on a blood agar plate (BAP) with 5 % sheep blood, chocolate agar, eosin-methylene blue agar (EMB agar), and a nutrient broth. The direct Gram-stained smear of the exudate showed 10–12 WBC per a high-power oil immersion field (x 1000) (Fig. 1***A***). After overnight incubation at 37 °C, bacterial growth was observed on the BAP and chocolate agar, but not on the EMB agar. The colonies on the BAP were very small, smooth, nonhemolytic, grayish-white (Fig. 1***B***), and the Gram-stained smear revealed Gram-negative coccobacilli (Fig. 1***C***). The catalase test and the oxidase test were positive. The bacterial isolate was identified as *P. canis* with 99 % probability by Vitek 2 compact (*bioMérieux, France*) using GN ID cards, and it was determined as *P. canis* biotype 1 based on indole production. The strain was ornithine decarboxylase positive and urease negative.

The antimicrobial susceptibility of the isolate was tested by the standard disk-diffusion method and Vitek 2 compact system using AST (antimicrobial susceptibility testing) cards. The results were interpreted according to the EUCAST guidelines, 2023 (https://www.eucast.org/clinical_breakpoints). The strain was susceptible to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ciprofloxacin, levofloxacin, doxycycline and trimethoprim-sulfamethoxazole.

Detailed and accurate identification of *P. canis* was performed by nucleic acid-based methods. A total DNA was extracted from blood agar cultures using a genomic DNA kit (*GeneProof, Czech Republic*). The strain was subjected to a 16S ribosomal RNA sequencing. A 918-bp fragment of the *rrs* gene was amplified using the 16S primers 5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-CCGTCAATTCMTTTRAGTTT-3′ as described previously [12]. The PCR product was purified using the Exo-CIPTM Rapid PCR-Cleanup kit (*New England Biolabs, USA*). Nucleotide sequencing of both strands of the PCR amplicon was performed

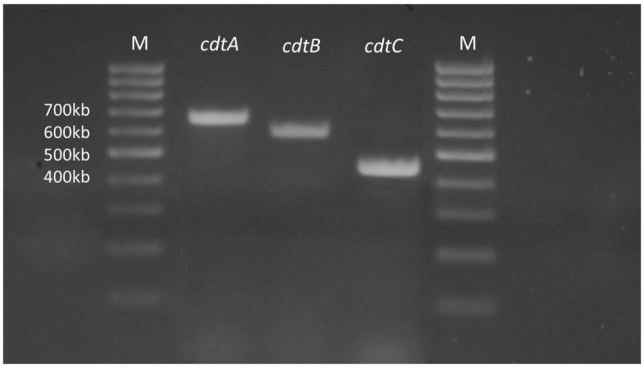

Fig. 2. PCR detection of cytolethal distending toxins in *P. canis* strain.

using an ABI 3500xl Genetic Analyzer (*Applied Biosystems, USA*). The NCBI bacterial database using BLASTn (http://blast.ncbi.nlm.nih.gov) revealed that the genome of the tested strain was 99.27 % identical to reference strain *P. canis* CIP:103294.

Our results were verified by a PCR amplification of a *sodA* housekeeping gene, that encodes manganese-dependent superoxide dismutase [12,13]. The specific detection of an 186 bp fragment confirmed the *P. canis* isolate.

Additionally, a PCR detection of common virulence factors in *P. canis* was carried out. The results revealed the presence of the three cytolethal distending toxins, *cdtA* – 693 bp, *cdtB* – 582 bp, and *cdtC* – 433 bp (Fig. 2). We optimize the annealing temperature at 53 °C for gene *cdtC* and the rest of the conditions are similar to the protocol of [14].

Discussion

P. canis is a classical pathogen associated with dog bites but it can also be isolated from infectious sites after bites from cats or other animals [2,3]. The review article of Mahony et al. [4] over 123 episodes of *Pasteurella* infections revealed *P.* canis (23.1 %) as second in frequency species after *P. multocida* (61.1 %).

The clinical forms of *P. canis* bite wound infections range from cellulitis and subcutaneous abscesses to arthritis, osteomyelitis, and bacteremia [3,7–9,11]. People with immunosuppression, underlying diseases such as diabetes, cirrhosis, and autoimmune disorders, were at greater risk for life-threatening invasive infections due to such pathogens [6]. Overall, the septic patients responded well to antibiotic treatment and the prognosis was favorable [8,9]. In studying *Pasteurella* infections over a twenty-year period, the detected mortality rate was at 1.0 % [4].

Herein, we described a 34-year-old man with *P. canis* soft tissue infection of the left hand after a cat bite. Our case is very similar to that reported by Kim et al. [7]. The authors presented wound infection following a dog bite in a 54-year-old woman without systemic diseases. The history of the bite, clinical symptoms, laboratory tests, surgical approaches, duration of hospitalization, and outcome were similar to our patient. For identification of the causative agent, Vitek 2 automatic system and 16 S rRNA gene sequencing were also used in their study, but the primers for amplification were different. Additionally, we detected a *sodA* housekeeping gene and cytolethal distending toxins (*cdtA*, *cdtB*, and *cdtC*) in *P. canis* strain.

Distinguishing of *P. canis* from closely related *Pasteurella species* is a challenge. Phylogenetic data based on 16S rRNA sequences and *sodA* gene sequences showed that *P. canis* is more closely related to *P. dagmatis* and *P. stomatis* than to *P. multocida* [13]. The presence of *cdtA, cdtB and cdtC* was considered potential *P. canis* characteristics, whereas their absence was typical for *P. multocida* strains [14].

In 2020, Zhu et al. [11] reported the first whole-genome sequence (WGS) of a strain *P. canis* isolated from a 75-year-old man with index finger osteomyelitis. They detected the antimicrobial resistance gene *qrnS1*, five virulence genes and one plasmid replicon. In 2023, Yoshida et al. [14] published comparative data of the virulence-associated genome characteristics of selected 10 *P. canis* WGSs and 16 *P. multocida* WGSs. The researchers found similar *cdtA-cdtB-cdtC* sequences in all *P. canis*, but in none of *P. multocida*.

Conclusion

This report presents a patient with *P. canis* soft tissue infection following an animal bite with emphasis on nucleic acid-based methods for laboratory diagnosis. Proper identification of etiological agents is important for treatment and management of such infections. According to our knowledge, this is the first report of *P. canis* in Bulgaria.

Ethical approval

The study was approved by the local ethics board of Medical University – Pleven. Data processing was anonymized and complied with local data protection legislation and with the European Directive on the Privacy of Data (95/46/EC).

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Author contributions

H.H and. P.H. contributed to the study conception and design. Material preparation and data collection and analysis were performed by H. H., A.A, P.Hand R.G. The first draft of the manuscript was written by H. H., and all authors commented on previous versions of the manuscript. The final review was done by H. H. and A.A. All authors also read and approved the final manuscript.

Consent

The patient provided written informed consent before being enrolled into the study.

CRediT authorship contribution statement

Hristina Y. Hitkova: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Alexandra S. Alexandrova:** Writing – review & editing, Formal analysis, Data curation. **Raina T. Gergova:** Formal analysis, Data curation. **Preslava M. Hristova:** Formal analysis, Data curation, Conceptualization.

Conflict of interest statement

The authors declare no conflict of interest.

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