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Short communication

Dogs as vectors of *Streptobacillus moniliformis* infection?

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Abstract

Rat bite fever is a bacterial zoonosis transmitted through the bite of rats. One of the two etiological agents that cause rat bite fever is *Streptobacillus moniliformis*. Rat bite fever is rare and very likely under diagnosed but occurs worldwide. Other animals, like dogs and cats that have mouthed a rat are often mentioned in the literature as potential risks for the attraction of rat bite fever. However, rat bite fever caused by the bite of a dog or cat has very seldom been documented. Therefore, to identify the possible risk for humans to become infected with *S. moniliformis* after having been bitten by a dog that has been in contact with rats, the presence of *S. moniliformis* in the mouth of these dogs was tested with molecular methods. Swabs taken from the mouth of 18 dogs with proven contacts with rats were tested for the presence of *S. moniliformis* DNA by PCR. An amplicon of the right size was obtained in 10 of the 18 dogs. Nucleotide sequencing of five amplicons of PCR positive samples demonstrated the presence of *S. moniliformis* DNA in the mouth of three dogs. A bite by these dogs therefore might infect humans with *S. moniliformis* and cause rat bite disease.

Keywords: *Streptobacillus moniliformis*; Rat bite fever; Dogs; Zoonoses; PCR

1. Introduction

Streptobacillus moniliformis is a highly pleomorphic, filamentous, Gram-negative non-motile and non-acid-fast rod (Levaditi et al., 1925). The rods often form filaments that occasionally show lateral bulbar swellings, which appear like a ''string of beads''. Hence the name moniliformis which means in the form of a necklace in Latin. The bacterium is a commensal in the mouth and pharynx of wild rats. Approximately, 50–100% of wild rats carry the organism (Elliott, 2007). In laboratory rats a similar percentage used to be noted before SPF animals were used. Nowadays *S. moniliformis* is occasionally detected in laboratory rodents (Boot et al., 2002). *S. moniliformis* can infect humans through a bite or scratch and by ingestion of food or water contaminated by rat excrements (the disease is than called Haverhill fever). Close contact with the oral flora of pet rats through kissing and sharing food has also been implicated as a cause of rat-bite fever (Albedwawi et al., 2006; Elliott, 2007). In humans, symptoms of rat bite fever include relapsing fever, rash, migratory polyarthralgias and vomiting. Occasionally rat bite

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fever can lead to pericarditis, endocarditis, myocarditis, meningitis, septic arthritis and focal abscesses (Elliott, 2007). The prevalence of *S. moniliformis* infections in humans is very likely underestimated. Only one in 10 rat bite incidences are reported. While it is known that *S. moniliformis* is transmitted to humans by rats and probably other rodents like mice, gerbils and squirrels, not much is known about a role of animals like dogs in infecting humans, but this possibility is invariably mentioned in text books. Approximately, 1 in 20 dogs will bite a human being during the dogs lifetime (Griego et al., 1995), but the number of proven cases of *S. moniliformis* infection after a dog bite is extremely small. In fact only in one report *S. moniliformis* infection as the result of a bite from a breed of dog (greyhound) that eats rats has been demonstrated in the last 30 years (Maynard et al., 1986; Peel, 1993). The involvement of dogs was likely in three other reports. Potential sources of infection in two young males with symptoms of rat bite fever (confirmed by a positive culture in one case) included common exposure to the same dog (MMWR, 1998). In a unique case of amnionitis involving *S. moniliformis* (Faro et al., 1980) the woman stated that the basement of her home was infested with rats or mice. Both family dogs were known to catch and kill the rodents and bring them in the living room. These dogs frequently licked the patient's hands and face and could have transmitted the infectious agent. Ditchfield et al. (1961) reported a case of *S. moniliformis* infection in a dog. The dog suffered from diarrhoea, vomiting, anorexia and arthritis in the hind legs. Post mortem examination showed the presence of *S. moniliformis*. To determine the possibility of transmission of *S. moniliformis* to humans by a dog bite the presence of *S. moniliformis* in the mouth of dogs has to be proven and was tested by PCR in this study.

2. Materials and methods

2.1. Animals

The buccal mucosae of 18 dogs living at 11 different locations in a rural environment were sampled using sterile cotton swabs. According to their owners all of these dogs had been in close contact with wild rats. Every dog was swabbed once by turning the wadding four times over the left and right buccal mucosae. Swab samples were placed in 1 ml of sterile 0.9% NaCl solution and transported to the laboratory where they were immediately analyzed.

2.2. PCR

Since it is difficult to isolate and identify *S. moniliformis* among the normal bacterial flora of the dog, using traditional bacteriological techniques a polymerase chain reaction (PCR) was applied to detect the bacteria in the obtained specimens. A 296 bp DNA fragment is amplified in this PCR, using a pair of primers based on the 16S rDNA gene described by Boot et al. (2002) (the forward primer: 5′-GCT TAA CAC ATG CAA ATC TAT-3′; the reversed primer: 5′-AGT AAG GGC CGT ATC TCA-3′). Samples were vortexed, the swabs were removed and the solutions were centrifuged for 10 min at 8000 × *g*. Pellets were resuspended in 50 μl milliQ water and heated for 10 min at 95 °C. The suspensions were then centrifuged for 10 min at 16,000 × *g* and the supernatant containing DNA was used for PCR analysis. DNA extracted from *S. moniliformis* CCUG 43797 (Culture Collection, University of Göteborg) was used as positive control. Amplicons were detected by electrophoresis in 1% agarose gel containing ethidium bromide (1 mg/ml), visualised in an UV transilluminator and photographed.

2.3. Cloning and sequencing

For further identification by nucleotide sequencing, PCR-positive samples were re-amplified for cloning. Amplicons were ligated into pGEM-T Easy vectors (Promega) and the plasmids were introduced into *Escherichia coli* DH5-alpha (NCCB 2955) by the heat-shock method. Plasmids with inserts were purified (Miniprep, QIAGEN) and send to BaseClear (The Netherlands) for nucleotide sequence determination.

2.4. Nucleotide sequence accession numbers

Nucleotide sequences obtained in this study have been assigned Genbank accession numbers from EU082089 to EU082093.

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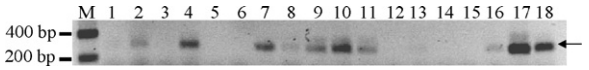

Fig. 1. Electrophoresis of the amplicons from the 18 salivary samples. M: DNA marker (SmartLadder, Eurogentec); arrow indicates the amplicon position.

3. Results

In Fig. 1 the result of the first amplification reaction is shown. Ten out of 18 samples were positive in the PCR (2, 4, 7, 8, 9, 10, 16, 17 and 18), albeit that amplicons with different intensities were obtained. This in fact was an unexpected high number. Therefore, for further identification by nucleotide sequencing, the PCR-positive samples were amplified again for cloning. In this second amplification, amplicons were obtained from only five samples (2, 7, 10, 17 and 18). Probably the DNA in the other five samples was degraded. Why this is the case we have not investigated. It was also not possible to obtain these five samples again, since contact with the owners of these five dogs was not possible. The five amplicons obtained in the second round of amplification were cloned into pGEM-T Easy vector and introduced in *E. coli* DH5-alpha. Analysis by nucleotide BLAST (Basic Local Alignment Search Tool) of the sequences using the NCBI database revealed that the nucleotide sequence from samples 2, 7 and 17 were identical with that of the *S. monoliformis* 16sRNA gene, while those from samples 10 and 18 were very similar to the sequences of *Leptotrichia* spp.

4. Discussion

Rat bite fever is usually associated with the bite of a rat and hardly ever with the bite of other animal species. In this study we have demonstrated, the presence of *S. moniliformis* DNA in the mouth of dogs, with known contacts with wild rats, by PCR. It is therefore very likely that *S. moniliformis* was also present in the mouth of these dogs. Although it was known that all dogs had been in contact with wild rats, a positive PCR in more than 50% of the dogs was considered as high. To exclude that all PCRs were false positive it was decided to clone and sequence the amplicons. False positive reactions with Leptotrichia species were found. Probably due to degradation of the DNA in the boiled lysates reamplification only gave a positive result in five of the 10 samples that were originally positive. Three of the five amplicons were indeed *S. moniliformis* DNA as judged from the nucleotide sequence. Even three out of 18 samples positive for *Streptobacillus* DNA is a rather high number. From these results one has to take into account that humans can be infected with *S. moniliformis* through a bite of dogs. Only a few reports have appeared in the literature on Streptobacillosis infection after the bite of a dog. One of the reasons might be that in the case of dog bites often antibiotic prophylaxis is given compared to reaction in case of rat bites. Since *S. moniliformis* is sensitive to most antimicrobials (Holroyd et al., 1988; Elliott, 2007) successful infection of the bitten individual might be prevented in this way. Furthermore it should be noted that without molecular methods isolated bacteria might have been misclassified.

Nothing is known about the infective dose for *S. moniliformis*, which can be different for different individuals, since two persons bitten by the same rat did not both get rat bite fever. It might therefore be that the number of *S. moniliformis* in the mouth of dogs or cats for that matter is normally not high enough to reach the infective dose needed in humans.

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