

# Systemic infection caused by *Klebsiella oxytoca* in a household Chinese hamster

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A female Chinese hamster with unknown age was referred for acute onset of anorexia, depression, and large cyst from the head to body. After referring, the patient died shortly. During necropsy, severe hemorrhage in the cyst, multiple mass on liver, and transformed right kidney were found. The infection was confirmed by cytology, cultures and PCR of 16S ribosomal RNA gene. This report describes a first case of naturally occurred systemic *Klebsiella oxytoca* infection in a household Chinese hamster.

**Key Words:** *Klebsiella oxytoca*, Chinese hamster, Systemic infection

## INTRODUCTION

*Klebsiella* spp. is a Gram-negative pathogen that are frequently isolated from various infections in animals and humans (Podschun and Ullmann, 1998; Gordon and FitzGibbon, 1999; Brisse and van Duijkeren, 2005). In human, *Klebsiella* spp. are increasing important opportunistic pathogens associated with severe nosocomial infections such as septicemia, pneumonia, and urinary tract infection (Brisse and Verhoef, 2001). In human, the infections are mainly caused by *K. pneumonia* and to a lesser degree by *K. oxytoca* (Podschun and Ullmann, 1998). Thus, mice and rats experimentally infected with *K. pneumoniae* have been used as models for infectious diseases (Baker, 1998). However, spontaneous infection of *Klebsiella* spp., especially *K. oxytoca* in rodents is very infrequent and there is no report in pet hamsters (Bleich et al, 2008).

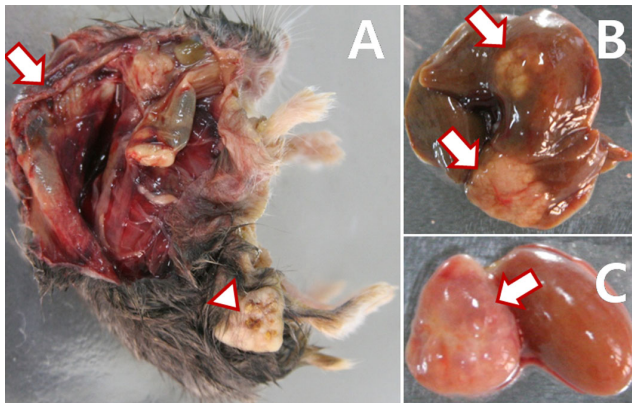
This report describes a case of natural *K. oxytoca* infection in a household Chinese hamster (*Cricetus griseus*).

## CASE

A female Chinese hamster with unknown age was referred due to acute onset of anorexia, depression, and expanded right side from the head to body. The clinical signs had been noticed about 2 weeks before presentation. The animal had not been treated before presentation. The hamster was housed on commercial sawdust bedding in a plastic box. The hamster had been living alone and had never been in contact with other animals. The hamster was fed commercial hamster food and water *ad libitum*.

At the first presentation, the hamster exhibited a large, fluctuating subcutaneous cyst on the right side from the head to body (Fig. 1), and well-circumscribed hairless masses on the outside of the right thigh and lower abdomen. The skins on the cyst and masses revealed complete alopecia. During physical examination, the hamster showed an offensive movement, however, its activity was significantly decreased. After all, the hamster died after the physical examination.

On necropsy, 13 ml of bloody exudate was collected



**Fig. 1.** The photo of the patient at necropsy showing (A) a large subcutaneous cyst filled with 13 ml bloody fluid on right side of the body (arrow) and skin mass (arrowhead), and (B) yellowish liver masses (arrow), and (C) transformed right kidney (arrow).

from the subcutaneous cyst by sterile fine-needle aspiration. The subcutaneous cyst was placed widely on the right side from the head to body. Mass formation or metastatic lesion was not examined in the cyst. In abdomen, multiple yellow masses were examined on the liver (Fig. 1). The right kidney was discolored (yellow) and transformed to round shape (Fig. 1). Impression smears were made from the cut surface of the liver and kidney.

The smears of the bloody exudate contained numerous intact and few phagocytosed erythrocytes. Leukocytes were occasionally observed, however, its number was not increased (not counted as too few). The cytologic diagnosis of the exudate was hemorrhagic effusion with unknown origin. The impression smears of the liver and kidney contain nucleated RBC, neutrophils and monocytes with numerous rods in common. Thus, we presumed that the lesions in the liver and kidney were caused by the bacterial infection.

In the meantime, the exudates collected from the cyst were cultured for bacteria at 37°C for 2 days in a Muller-Hinton agar in aerobic and anaerobic conditions. Under both conditions, the cultured plate showed growth of gram-negative rods. Bacterial identification using remel Rapid™ one kit (remel, Lenexa, KS, USA) showed that the gram-negative rods are *K. oxytoca* in both cultures.

To further investigate the possibility of the infection by *K. oxytoca* in the liver and kidney, molecular diag-

nosis was performed. The genomic DNA was isolated from the exudate using a Dynabeads® DNA Direct™ Universal kit (Invitrogen, Carlsbad, CA, USA). The genomic DNAs encoding the 16S ribosomal RNA (16S rRNA) gene were amplified using the primers 16SrRNA27F and 16SrRNA1492R as described previously (Vallianou et al, 2008). The PCR amplifications were performed in a total volume of 50 µL. The final reaction conditions were as follows: 50 mM KCl, 10 mM Tris-HCl (pH 8.3, 25°C), 1.5 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 100 ng of each primer, and 5 units of Taq polymerase. The PCRs were performed in a TaKaRa Thermal Cycler Dice (Takara Bio Inc., Otsu, Shiga, Japan) under the following conditions: an initial denaturation at 94°C for 2 min 30 s, followed by 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 45 s, and a final run at 72°C for 5 min. The PCR products were separated by electrophoresis for 50 min at 100 V in a 2% agarose gel and were stained with ethidium bromide for visualization under ultraviolet light. The amplicons were sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (PE Applied Biosystems, Foster City, CA, USA). A comparison with the gene sequences deposited in GenBank revealed that the 16S rRNA gene sequences of all isolates were 100% similar to the *K. oxytoca* sequence that had been deposited in Japan (GenBank accession number AB244452). These results, together with those of the cytology and cultures, were suggestive of *K. oxytoca* infection in the liver and kidney, indicating systemic *K. oxytoca* infection.

Antibiotic susceptibility testing of the cultured *K. oxytoca* was performed by disc diffusion method using the discs of amikacin, ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, cefaclor, cefazolin, cefotaxime, ceftriaxone, cephalothin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, tetracycline, trimethoprim/sulfamethoxazole, doxycycline, and ciprofloxacin (BD, Franklin Lakes, NJ, USA) at 37°C for 3 days in aerobic condition. Consequently, amikacin, ampicillin/sulbactam, cefaclor, cefotaxime, cephalothin, chloramphenicol, gentamicin, trimethoprim/sulfamethoxa-

zole, doxycycline, and ciprofloxacin were confirmed as susceptible antibiotics.

## DISCUSSION

This report describes a natural infection of *K. oxytoca* in a household Chinese hamster. At first presentation, the expanding lesion from the head to body was presumed as a metastatic lesion because of two similar-shaped skin masses on thigh and lower abdomen. On necropsy and culture, the expanded lesion was, however, filled with septic bloody fluid that is likely to be induced by hemorrhage. On bacterial identification, same *K. oxytoca* was isolated from the lesions. Thus, the patient was diagnosed as systemic *K. oxytoca* infection, and a hemorrhage shock with septicemia was presumed as a cause of the death. This report is the first to show that hamsters can be naturally infected by *K. oxytoca*.

Based on their oxygen requirement, bacteria can be separated into obligate aerobes, obligate anaerobes, facultative anaerobes, microaerophiles, and aerotolerant anaerobes (Nester et al, 2001). Enterobacteria including *Klebsiella* spp. belongs to facultative anaerobes that can grow in aerobic and anaerobic conditions. Facultative anaerobes use aerobic respiration if oxygen is available, but use fermentation or anaerobic respiration in its absence (Bott, 1997). Growth is more rapid when oxygen is present because aerobic respiration yields the most ATP of all these processes (Kovtunovych et al, 2003). In this report, isolated *K. oxytoca* revealed different colony forming time; 1 day in aerobic condition and 3 days in anaerobic condition. It suggests that the time more than 3 days should be needed for the diagnosis of infection based on the anaerobic culture in suspected enterobacteria-infected patients.

The taxonomic status of *K. oxytoca* has been debated, ranging from interpretation to consider it as another species or subspecies of *K. pneumoniae* (Kovtunovych et al, 2003). By the predominantly used classification of Orskov and recent molecular phylogenetic analyses confirmed that *K. oxytoca* is a separate species of the

*Klebsiella* genus (Boye and Hansen, 2003). However, a distinction between *K. oxytoca* and *K. pneumoniae* is a difficult task by routine microbiologic equipments because of their similar properties. Therefore, we performed the PCR and direct sequencing procedures for identification, and we confirmed that the sequences were 100% similar to the sequence of *K. oxytoca*.

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## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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