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Detection of Drug-Resistant *Klebsiella pneumoniae* in Chinese Hares (*Lepus sinensis*)

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ABSTRACT: We investigated an outbreak of acute pneumonia among adult Chinese hares (*Lepus sinensis*) and diarrhea among juvenile hares in Hebei Province, China, in 2012. Diagnosis was based on necropsy examination, microbial characteristics, biochemical identification, and nucleotide sequence analysis. The isolated bacteria from tissue samples of dead hares were identified as *Klebsiella pneumoniae* ssp. *pneumoniae* (*K. pneumoniae*). This *K. pneumoniae* was resistant to the antimicrobials imipenem, meropenem, penicillin, and vancomycin, but was highly sensitive to cefepime, cotrimoxazole, and enrofloxacin. *Klebsiella pneumoniae* is an important opportunistic pathogen, which often causes nosocomial infections in immunocompromised patients. However, the emergence of drug-resistant *K. pneumoniae* in hares indicates the existence of increasing risk of pathogen transmission between humans and wildlife. Given the close association between wildlife, livestock, and humans, it is important to identify epidemiologic factors associated with infection in these hares to minimize the risk of *K. pneumoniae* transmission.

Key words: Acute pneumonia, Chinese hares, diarrhea, *Klebsiella pneumonia*.

Recently, the US National Institutes of Health Clinical Center experienced an outbreak of carbapenem-resistant *Klebsiella pneumoniae* that affected 18 patients, 11 of whom died (Snitkin et al. 2012). Over the past 12 yr, *K. pneumoniae* has been detected in clinical isolates worldwide, including India (Snitkin et al. 2012), the United Kingdom (Kumarasamy et al. 2010), South America (Villegas et al. 2006), France (Naas and Nordman 2005), the US (Woodford et al. 2004), Italy (Luzzaro et al. 2004), and Singapore (Koh et al. 1999).

Klebsiella pneumoniae can cause pneumonia, septicemia, urinary tract infections, and meningitis in humans. The signs of *K. pneumoniae* infection in animals vary, depending on species. Pneumonia and septicemia were associated with *K. pneumoniae* outbreaks in humans as well as primates and some wild animals, such as vervet monkeys (*Chlorocebus aethiops sabaeus*; Whitehouse et al. 2010), sika deer (*Cervus nippon*; Wang et al. 2009), New Zealand sea lions (*Phocarctos hookeri*; Castinel et al. 2008), and silvery gibbon; *Hylobates moloch*; Chen et al. 2007).

An outbreak of acute pneumonia among adult Chinese hares (*Lepus sinensis*) and diarrhea among juvenile hares in Hebei Province, China was reported by local residents to the National Research Center for Wildlife Born Diseases (NCWBD) at the Institute of Zoology (IOZ), Chinese Academy of Sciences (CAS), in early 2012. Sick adult hares displayed depression, inappetence, fever, sneezing, rhinorrhea, labored respiration, abdominal distension, and black mushy excrement, and died within 1–2 days. The juvenile hares experienced severe diarrhea and quickly died, while pregnant hares had abortions. Dead hares were collected and transported immediately to the NCWBD for necropsy. Tissue samples from dead hares were used for bacterial culture to identify pathogens.

Of 10 randomly selected and tested sick and dead hares, eight were positive for *K. penumoniae*. Moreover, 90% of the positive hares showed tracheal bleeding, purulent pneumonia with consolidation of congestion, bleeding and festering of

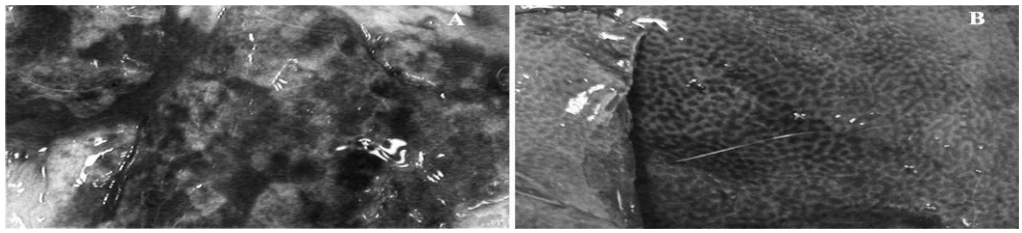

FIGURE 1. Pathologic changes of lung and liver in Chinese hares (*Lepus sinensis*) from Hebei Province, China, infected with *Klebsiella pneumoniae*, 2012. (A) The lung contained serious abscesses, bleeding, and passive congestion. (B) The liver contained extravasated blood and swelling with acicular focal necrosis.

the lung, extravasated blood, and swelling with acicular focal necrosis in the liver (Fig. 1). In chronic cases in which duration was >1 wk, we found suppurative pleurisy, granulomatous lesions in the lung essence, and gray nodules on the lung surface. The small intestine had ulcerative lesions, the cecum serous was filled with gas, and intestinal contents emerged as brown paste or water. Microscopically, many pulmonary alveolar septa were thickened due to cell proliferation and cellular infiltration.

Bacterial growth was observed on lysogeny broth agar and blood agar plates. Colonies were isolated from tissue samples of trachea, lung, liver, and intestine. Gram-negative colonies were processed for PCR with the use of universal primers to amplify the 16S RNA gene, and products were purified and used for DNA sequencing. The sequences were compared to bacterial *K. penumoniae* sequences available in GenBank. Our sequences were submitted to GenBank as accessions JX512365–JX512367. Results suggested that *K. pneumoniae* was the major pathogen causing pneumonia and death of the hares. The organism we isolated was confirmed biochemically with the use of the BD Phoenix™ 100 (Maryland, USA); the final identification of the bacterium was *Klebsiella pneumoniae* ssp. *pneumoniae*. These bacteria were isolated from hares that had died from acute infections as well as those that experienced chronic infections.

To test whether the isolated *K. pneumoniae* could cause death in experimental rabbits the same way as the hares, four specific-pathogen-free rabbits (Japanese White Rabbit [*Oryctolagus cuniculus*]; Beijing Vital River Experimental Animal Technology Limited Corporation, Beijing, China) were divided randomly into two groups. Guidelines for Ethical Conduct in the Care and Use of Experimental Animals were followed and the experimental procedures were approved by the Ethics Committee on Animal Experimentation of the Institute of Zoology, Chinese Academy of Sciences. Two rabbits in each group were inoculated intraperitoneally with either 0.5 mL (1×10^6 colony-forming units [CFU]/mL) of pure bacterial culture or 0.5 mL of phosphate buffer, respectively. The two experimental rabbits died within 24 hr after inoculation, while the two control rabbits appeared normal. On necropsy, the pathology of the two rabbits that died was similar to that of the hares, histopathology was consistent with the same diagnosis as the hares, and a bacterium identical to that used for the inoculation was detected in tissues from the dead rabbits.

Twenty antibiotics were used for antimicrobial susceptibility testing. *Klebsiella pneumoniae* was resistant to imipenem, meropenem, penicillin, and vancomycin, but highly sensitive to cefepime, cotrimoxazole, enrofloxacin, and others (Table 1). Thus, the *K. pneumoniae* was not only resistant to ordinary antibiotics, but also to

TABLE 1. Antibiotic susceptibility of *Klebsiella pneumoniae* isolates from Chinese hares (*Lepus sinensis*) compared to isolates from humans.

Antibiotic	Hare isolates	Human isolates
Imipenem	Resistant	Sensitive
Meropenem	Resistant	Sensitive
Penicillin	Resistant	Resistant
Vancomycin	Resistant	Resistant
Polymyxine B	Resistant	Sensitive
Ampicillin	Resistant	Resistant
Neomycin	Minimally sensitive	Sensitive
Norfloxacin	Moderately sensitive	Sensitive
Sulfafurazole	Highly sensitive	Resistant
Streptomycin	Minimally sensitive	Sensitive
Kanamycin	Moderately sensitive	Sensitive
Cefepime	Highly sensitive	Moderately sensitive
Chloramphenicol	Highly sensitive	Sensitive
Ciprofloxacin	Highly sensitive	Resistant
Enrofloxacin	Highly sensitive	Sensitive
Cotrimoxazole	Highly sensitive	Resistant
Amikacin	Highly sensitive	Moderately sensitive
Furazolidone	Moderately sensitive	Sensitive
Gentamycin	Moderately sensitive	Sensitive
Tobramycin	Moderately sensitive	Sensitive

carbapenem antibiotics. When we compared antibiotic susceptibility of *K. pneumoniae* isolated from hares to isolates from humans, we found differences in susceptibility. It is possible that these differences were due to strain variation resulting from interspecies transmission.

Our results confirmed that drug-resistant *K. pneumoniae* was responsible for the outbreak in hares. However, the source of this drug-resistant *K. pneumoniae* is unclear. Factors such as expansion of human activities and contamination of the environment with human wastes may play a role in the spread of the pathogen to migratory wildlife. *Klebsiella penumoniae* infection in wildlife may be interpreted as an indicator of a high level of environmental contamination with the bacterium. *Klebsiella pneumoniae* is widespread in mammals and in the environment, and it is an opportunistic pathogen causing disease when other factors lower host defenses (Snyder et al. 1970).

There are at least three possible scenarios that could explain the transmission of *K. pneumonia* to hares. Firstly, the hares may have become infected via environmental exposure to *K. pneumonia* in hospital waste. Secondly, with the increase of human activities, it was possible that *K. pneumoniae* was transmitted from humans to hares. It is also possible that the pathogen was transmitted to hares from wildlife. To reduce the risk of zoonoses, the public should be educated about the risks associated with wildlife, livestock, and humans, and proper surveillance should be implemented.

Incidents of mortality in hares caused by *K. pneumoniae* have been reported only rarely in China (Ren et al. 2010; Wang et al. 2012). The emergence of drug-resistant *K. pneumoniae* in hares suggests that *K. penumoniae* can be transmitted between humans and wildlife and that free-living hares may pose a risk of zoonotic transmission. Surveillance in wildlife populations could help monitor wildlife health in this region. Further investigation is required to understand the epidemiology of *K. pneumonia* infection better and to reduce the risk of emerging infectious diseases.

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