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CASE REPORT
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Acute Death of Kangaroos in a Zoo Due to Highly Pathogenic *Corynebacterium pseudotuberculosis* and *Yersinia pseudotuberculosis*

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ABSTRACT

***Background*:** "Lumpy jaw" is disease effecting wallabies and kangaroos, particularly in *Macropus rufus* and *Macropus giganteus*. In the most serious situations, additional tooth loss and fistulas follow, accompanied by a stench, weight loss, and eventually death due to sepsis or blood poisoning. "Lumpy jaw" disease has seriously affected the normal display and health of kangaroos, and cause a huge economic loss. There was an outbreak of jaw infection in kangaroos at the Hongshan Forest Zoo. Two *Macropus giganteus* and two *Macropus rufus* died of "lumpy jaw". The main objective of the describing case was to isolate pathogens, provide a basis for follow-up treatment, and serve to establish a disease prevention protocol. ***Case*:** Four grown-up kangaroos (two *Macropus giganteus* and two *Macropus rufus*) were raised in Hongshan Forest Zoo, which had obviously clinical symptoms, such as oral lesions of pus, necrotic tissue, rotting teeth, then died of "lumpy jaw". Oral swab samples were collected from the lesion sites of the dying kangaroos. Mice experiments were conducted to examine the pathogenicity of the strains. Tests of antimicrobial susceptibity were performed to prescribe with better drug treatments for kangaroos. *Corynebacterium pseudotuberculosis* and *Yersinia pseudotuberculosis* were identified based on morphology, culture characteristics and biochemical tests. *Corynebacterium pseudotuberculosis* (G^+) in Sucrose, Mannitol, Lactose, Maltose, Glucose tubes were positive, that acids and gases both production, in Gelatin liquefaction, Indol test, MR were positive, that only acids production, others were negative; *Yersinia pseudotuberculosis* (G^-) in Urea, MR were positive, that only acids production, others were negative.The infected mice presented with gum erosion or ulcers when the two pathogens were injected subcutaneous at the oral regional by 2-3 point at 0.2 mL of individual strains 1.0×10^9 CFU/mouse. Drug sensitivity tests showed that *Corynebacterium pseudotuberculosis* is highly sensitive to Erythromycin and Sulfamethoxazole; meanwhile, *Yersinia pseudotuberculosis* is highly sensitive to Sulfamethoxazole, Nitrofurantoin, Penicillin-G and Erythromycin. The other "lumpy jaw" kangaroos in the zoo now receive oral screening with antibiotics. ***Discussion*:** *Yersinia pseudotuberculosis* is one of three pathogenic bacteria of the gram-negative genus *Yersinia*; it can be found in birds and other mammals. It also spreads through soil, plants, and insects in the environment. *Yersinia pseudotuberculosis* can also lead to fatal systemic symptoms in human. *Corynebacterium pseudotuberculosis* belongs to the *Actinobacteria* family, which are associated with *Caseous lymphadenitis* in breeding animals, especially in goats and sheep. The isolates affected the lymph nodes and visceral organs of the kangaroos in this case, who presented with gingivitis or stomatitis. It is the first report of *Corynebacterium pseudotuberculosis* and *Yersinia pseudotuberculosis* co-infecting kangaroo and causing a fatal case of "lumpy jaw" in China. In this case, isolation and identification of pathogenic bacteria were carried out on the sick kangaroos, and animal test and drug susceptibity test were conducted. The study results could provide theoretical basis for the follow-up treatment and prevention method of this disease.

Keywords: kangaroo, *Yersinia pseudotuberculosis*, *Corynebacterium pseudotuberculosis*, mice, drug susceptibity.

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INTRODUCTION

"Lumpy jaw" is disease effecting wallabies and kangaroos, particularly in *Macropus rufus* and *Macropus giganteus* [16]. The early clinical features include salivating, jaw swelling, depression, and loss of appetite in adult animals. Additionally, the swelled area increases suddenly and may cover the whole face, and can include purulent secretions. In the most serious situations, additional tooth loss and fistulas follow, accompanied by a stench, weight loss, and eventually death due to sepsis or blood poisoning. Pordy *et al*. [10] termed this serious jaw swelling as "lumpy jaw". In this case, pathogens from the oral lesions of dying kangaroos at the Hongshan Forest Zoo were isolated and studied. This serves as an update on the primary etiologic agent for lumpy law in captive macropods, provides a basis for follow-up treatment, and serves to establish a disease prevention protocol.

CASE

Four kangaroos (two *Macropus giganteus* and two *Macropus rufus*) were raised in Hongshan Forest Zoo, who had obviously clinical symptoms (i.e., oral lesions of pus, necrotic tissue, and rotting teeth). The sick animals got thinner and presented with a low fever. Anorexia occurred due to swelling of the gums in all sick kangaroos a month ago and died of multiple organ failure very soon (Figure 1a). We observed the classical symptoms of scurvy, including spongy gums, loosening of the teeth, and bleeding into the skin and mucous membranes. The distribution of particles in osteolytic tissue (Figure 1b) and the bone marrow necrosis (Figure 1c) inside the tooth were apparent. The pus of oral lesions, serum samples, lymph nodes, and rotting teeth were placed on microscope slides and incubated on TSA containing 5% (v/v) sheep blood[1] at 37ºC overnight or in an atmosphere of 10% CO_2 for 48 h.

Brown colonies were observed in a Gram stain under a microscope. The two pure cultures were observed under microscope after being Gram stained. One was Gram positive with single, short chain, or long catenation, about 1.5-5 μm in diameter, with unevenly dyeing, no flagellum, no spores, and no capsule (Figure 2). The other is Gram negative with ovoid or short rods, scattered within the vision, short but straight, and do not produce spores or capsules. The strains were identified as *Corynebacterium pseudotuberculosis* (G^+) and *Yersinia pseudotuberculosis* (G^-).

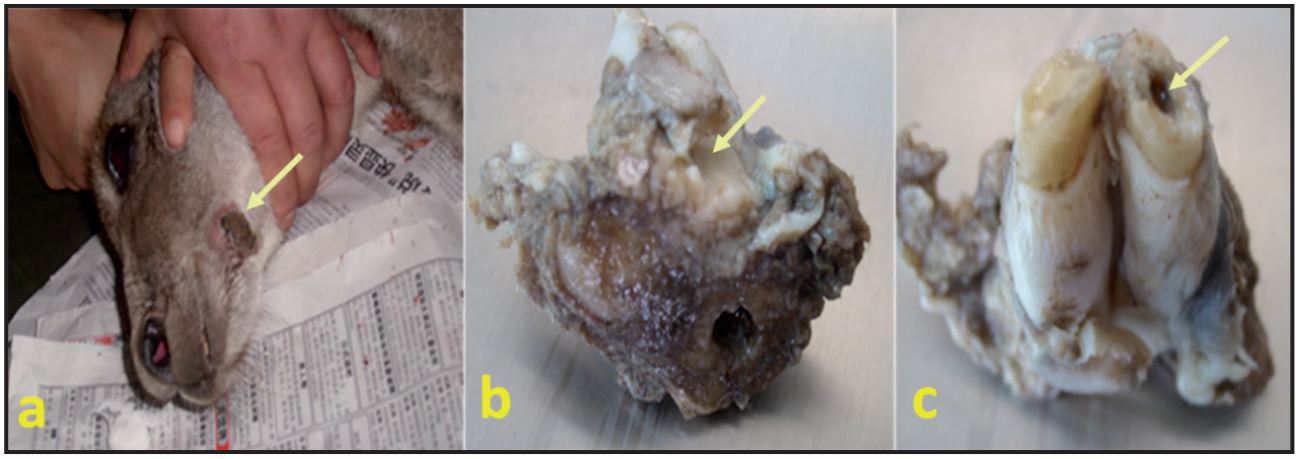

Figure 1. Pathological lesion in kangaroos. A- Skin Ulcer on the submandibular (Yellow arrow), being severe trauma. B- Tooth osteomyelitis (yellow arrow). C- Tooth decay (yellow arrow) in kangaroos.

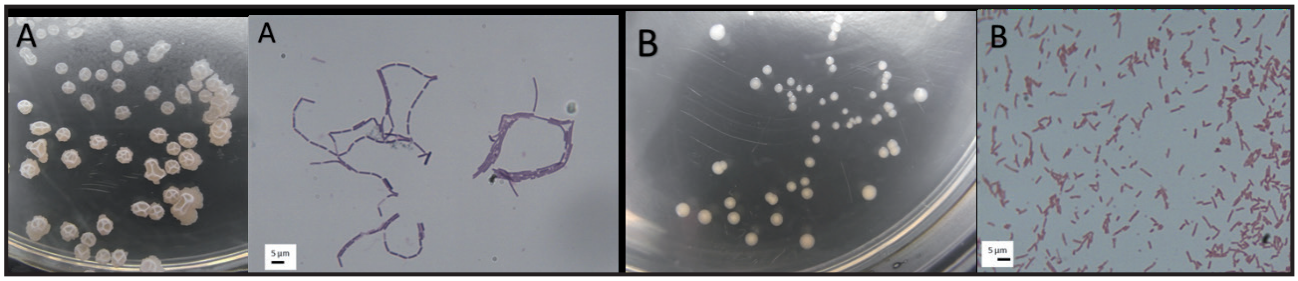

Figure 2. Colony morphologies of isolates from the oral swab samples in the dying kangaroos. A- The colony morphology of Gram-positive stain, single, short chain or long catenation, about 1.5~5 μm, unevenly dyeing, no flagellum, no spores and no capsule. B- The colony morphology of Gram-negative stain, ovoid or short rods, scattered, short but straight, and no spores and no capsule.

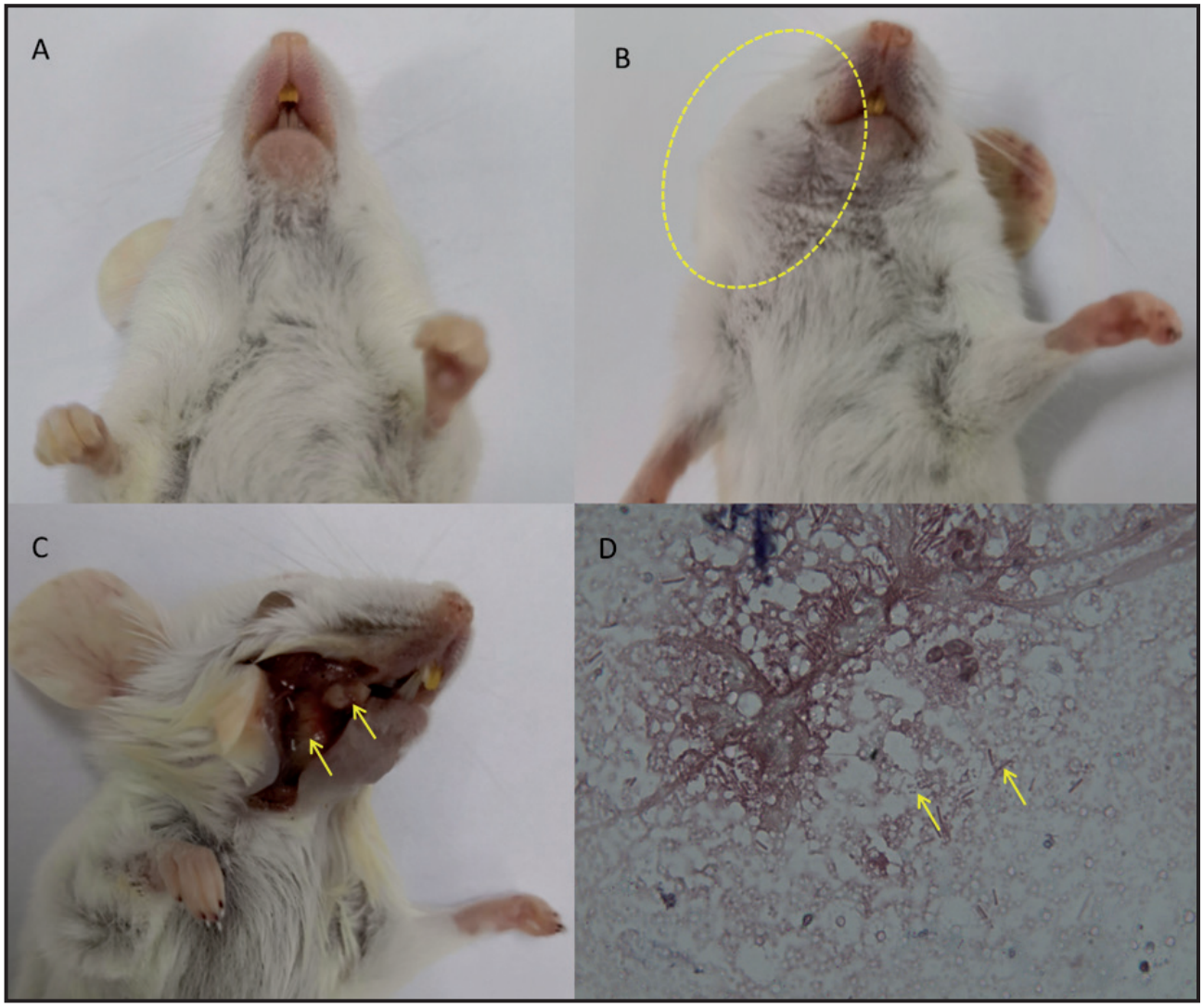

Figure 3. Submandibular lesions of death mice. A- In control group, Submandibular is normal after subcutaneous injection with PBS. B- Abscesses on the submandibular (dotted yellow line) of the mouse in co-infection group. C- Inflammatory exudates and tooth lesion (yellow arrows) in mouse in co-infection group. D- The pus with methylene blue staining showed the short rods germs and typical rod-shaped bacteria (yellow arrows).

Four-week BALB/c mice (n=12) from the Comparative Medicine Center of Yangzhou University were housed in filter top cages with free access to food and water under a 12-hour light/dark cycle. Mice were randomly divided into four groups (n=3 mice per group). Bacterial cells were washed twice with sterile phosphate buffer solution (PBS), and the cells were diluted to a concentration of 1.0×10^9 CFU/ mL for inoculation. The mice were inoculated by subcutaneous injection with 0.2 mL of individual strains (two groups), mixed strains (third group), or PBS (fourth group).

Following oral infection with *Yersinia pseudotuberculosis*, the mice developed lumpy jaw, which lasted for 4 days. One mouse died in the *Yersinia pseudotuberculosis* group, two mice died in the co-infected group, and no mice died in the *Corynebacterium pseudotuberculosis* group or the control group. The infected mice showed classical symptoms of "lumpy jaw", such depression, anxiety, and jaw swelling. The pulp chamber of the dying mice was enlarged compared with the control, and air pockets could be observed in some severe cases of infection. The co-infected group exhibited more symptoms than the single infection groups.

A total of 14 kinds of micro biochemical tubes[2] (Sucrose, Mannitol, Lactose, Maltose, Glucose, Citrate, Hydrogen sulfide, Urea, Odc, Esculin, Inositol, Salincin, Arabinose, Gelatin liquefaction) were used for detection. The growth phrase of the culture were measured and compared between isolates. Bacterial motoricity, Indole, and Methyl red (MR) /VP tests were performed according to standard protocols.

Two strains of pure culture inoculate microbiological control, peptone water, and glucose peptone water for 48 h to biochemical identification. *Corynebacterium pseudotuberculosis* (G^+) in Sucrose, Mannitol, Lactose, Maltose, Glucose tubes were positive, that acids and gases both production, in Gelatin liquefaction, Indol test, MR were positive, that only acids production, others were negative; *Yersinia pseudotuberculosis* (G^-) in Urea, MR were positive, that only acids production, others were negative.

The strains were inoculated on TSB[3] plates and colonies were coated on bacterial sticks. Various drug sensitivity disks[2] were attached to the surface of the medium using sterile forceps (Amoxicillin, Rifampicin, Macrodantin, Penicillin-G, Streptomycin, Erythromycin, Amikacin, Ciprofloxacin, Gentamicin, Kanamycin, Ofloxacin, Doxycycline, Tetracycline, Cefoperazone, Cefalexin, SMZ-TMP, Nitrofurantoin and Norfloxacin). The sphere of inhibition was measured after incubation at 37ºC for 24 h.

Drug sensitive testing revealed that *Corynebacterium pseudotuberculosis* was highly sensitive to Erythromycin (E) and SMZ-TMP; moderately sensitive to Amikacin, Kanamycin, Gentamycin, and Streptomycin; resistant to Penicillin-G, Nitrofurantoin, and Rifampicin. *Yersinia pseudotuberculosis* was highly sensitive to SMZ-TMP, Nitrofurantoin, Penicillin-G, and Erythromycin; moderately sensitive to Amikacin, Kanamycin, Rifampicin, and Gentamicin; and resistant to Streptomycin.

DISCUSSION

In recent years, reports of "lumpy jaw" in the wild have reduced gradually; however, cases in domestic zoos have been on the rise. "Lumpy jaw" in kangaroos is one of the most serious diseases threatening kangaroos. In 1978, Taylor *et al*. [16] confirmed that "lumpy jaw" is caused by *Nocardia* spp. and *Bacteroides ruminicola* var. *brevis*, either individually or in concert. In kangaroos, *Actinomyces*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Necrosis bacillus* have been reported to be the etiologic agent [4,7]. There are some other bacteria, such as *Streptococcus* spp. and *Bacteroides* spp. that had been isolated from captive macropods [1,14]. *Yersinia pseudotuberculosis* is one of three pathogenic bacteria of the gram-negative genus *Yersinia*; it can be found in birds [5], dogs [19], calves [13], cockatoo [12], fox [2] and cows [8], among others [17]. It also spreads through soil, plants, and insects in the environment [6]. *Y. pseudotuberculosis* can also lead to fatal systemic symptoms in human [11,15,18]. *Corynebacterium pseudotuberculosis* belongs to the *Actinobacteria* family, which are associated with *Caseous lymphadenitis* in breeding animals, especially in goats and sheep [3,9]. The isolates affected the lymph nodes and visceral organs of the kangaroos in this case, who presented with gingivitis or stomatitis. Unbalance diet includes soft food with low roughage content and coarse feed pellets more likely result in lumpy jaw, the primary food for the animals in this case, was potentially an important vector for transmission of infections in kangaroos.

In this case, isolation and identification of pathogenic bacteria were carried out on the sick kangaroos, and animal test and drug sensitivity test were conducted. The study results could provide theoretical basis for the follow-up treatment and prevention method of this disease.

MANUFACTURERS

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