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Animal Diseases

CASE REPORT

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Isolation and identification of four pathogenic bacterial strains from edible snake (*Elaphe carinata* and *Ptyas mucosus*) farms with pneumonia in China

Ying Xia, Siyu Long, Yuxuan Peng, Songkang Qin and Yaoqin Shen*

Abstract

This report describes an outbreak and treatment of pneumonia and enteritis in a snake farm with more than 3000 snakes containing *Elaphe carinata* (one-year-old) and *Ptyas mucosus* (three-month-old) seedlings in Huanggang, Hubei, China. Gentamicin was used once in the early stage as treatment, administered orally with water or feed by owners, but mortality increased. Lobar pneumonia was confirmed by dissection and histopathology in infected snakes. Four main pathogenic bacteria were isolated and identified with culture and 16S rRNA sequencing: *Staphylococcus sciuri*, *Salmonella enteritis*, *Vagococcus fluvialis* and *Providencia vermicola*. Drug susceptibility tests were performed, and amikacin, gentamicin and cefitriaxone were chosen accordingly. After two rounds of treatment, the clinical signs for *Elaphe carinata* were under control, and the mortality was close to 0% after treatment. However, treatments for *Ptyas mucosus* seedlings did not work well, potentially because of poor administration technique and weak body condition.

Keywords: *Staphylococcus* sciuri, Antimicrobial resistant, Edible snakes, Dermatitis

Introduction

Both King rat snakes (*Elaphe carinata*) and Oriental rat snakes (*Ptyas mucosus*) are numerous in the wild. They are the main breeds of edible snakes raised in China at present. Due to the lack of experience of farmers and improper breeding conditions, various snake diseases frequently occur. Pneumonia is a common disease caused by improper feeding and management, and its incentives may include poor environmental hygiene and large changes in temperature and humidity (Ackerman 1998; Pees et al. 2007). Common causes of snake pneumonia include bacterial, fungal, viral and parasite infections. It may also be due to noninfectious factors, such as aspiration and tumors (Comolli et al. 2021). Bacterial pneumonia in snakes is more common. Pneumonia can be diagnosed by history and clinical symptoms, combined with imaging and histopathological examinations. When pneumonia occurs, blind empirical medication is usually not advisable, and blind use of broad-spectrum antibiotics can lead to drug resistance. Oral and nasal secretions or lung tissue samples should be collected from infected snakes. The collected samples can be submitted for bacterial isolation, culture, identification and drug susceptibility testing, which can guide clinical treatment. In exotic companion animal veterinary clinics, snakes can be examined with endoscopes for pulmonary evaluation and sample collection (Stahl et al. 2008; Knotek and Jekl 2015). Given appropriate environmental conditions and treatment with appropriate antibiotics, the prognosis of snake pneumonia is usually good.

*Correspondence: yshen@mail.hzau.edu.cn

College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

Fig. 1 Clinical signs. **A** Hematemesis for more severely infected snakes; **B** Full mouth of greenish purulent secretion during examination

This study is the first report to isolate *Providencia* spp. and *Vagococcus fluvialis* from snakes and reptiles. Additionally, this is the first report on *Staphylococcus sciuri* infection in snakes in China.

Case presentation

Case history

The case was received from an edible snake farm in Xishui County, Huanggang city, Hubei Province, China, in October 2019. This farm has raised more than 2,000 one-year-old *Elaphe carinata* and nearly 1,000 *Ptyas mucosus* seedlings. Since the beginning of summer in July 2019, some snakes have shown clinical signs of lethargy, anorexia and poor growth rates. Morbidity and mortality increased over time. In October 2019, the morbidity of *Elaphe carinata* reached 30%, and among those, mortality was approximately 80%. Snakes were raised in separate rooms according to different species and ages. Each room is 4×5×3 m in size, and the floor was paved with sand and stones. With floor heating, the room temperature was controlled within 27–29°C, and no humidification equipment was set up. There was no disinfection area in the entrance, and no disinfection procedures were taken, which led to potential infectious disease transmission. Healthy snakes were fed with chicken, while sick snakes were force fed with chicken mince. The gavage tube was cross-used for anorexic snakes, and no sterilizing procedures were taken between each feeding, which potentially sped up infections.

Clinical findings

Infected snakes presented with lethargy, anorexia, lean body condition, heavy breathing, full mouth of greenish purulent secretion (Fig. 1A) and loose stool. More severe individuals showed hematemesis once a while (Fig. 1B).

Pathological dissections were performed for infected snakes in the field. Gross appearance showed pulmonary hyperemia and edema. Purulent mucous secretion was seen between lung tissues (Fig. 2B and C), and the normal pink reticular lung structure was damaged. Infected lung tissues could not be smoothly separated from the body wall. The liver was swollen and yellowish in appearance. Watery feces were seen in the intestines.

Specimen preparation

The lungs and livers of 5 sick king snakes and 5 oriental rat snakes were collected, stored on ice and transported to the veterinary teaching hospital of Huazhong Agricultural University in Wuhan, Hubei Province. The collected materials were fixed with 10% formaldehyde and embedded into wax blocks in appropriate pathological sections.

Pathogens from collected tissues were isolated and cultured on TSA (tryptose soya agar) plates. Pathogenic bacteria were identified with bacterial morphology observation and 16S rRNA sequence alignment analysis.

Pathological findings

Slides showed degeneration and necrosis of lung tissues and steatosis of hepatocytes (Fig. 3). The isolated pathogenic bacteria were Staphylococcus sciuri, Salmonella enteritis, Vaginalococcus fluvialis and Provencia vermicola. Drug susceptibility tests were performed for each strain, and the results are shown in Table 1.

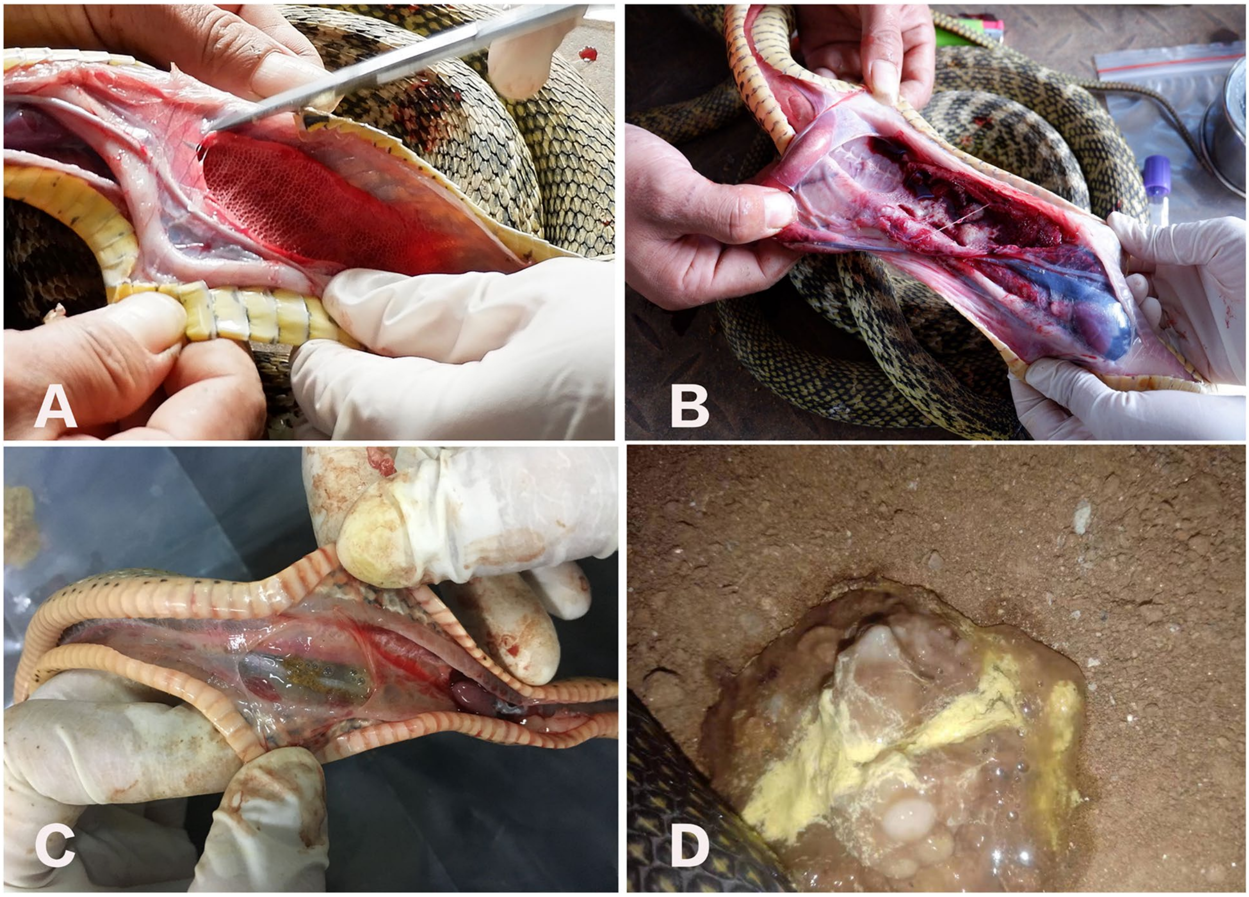

Fig. 2 Field dissections. **A** Normal pink reticular lung tissue in *Elaphe carinata*; **B** Purulent mucous secretion seen in damaged lung tissue in infected *Elaphe carinata*; **C** Greenish exudate seen in between damaged lung tissue in infected *Ptyas mucosus*; **D** Watery diarrhea seen in *Elaphe carinata*

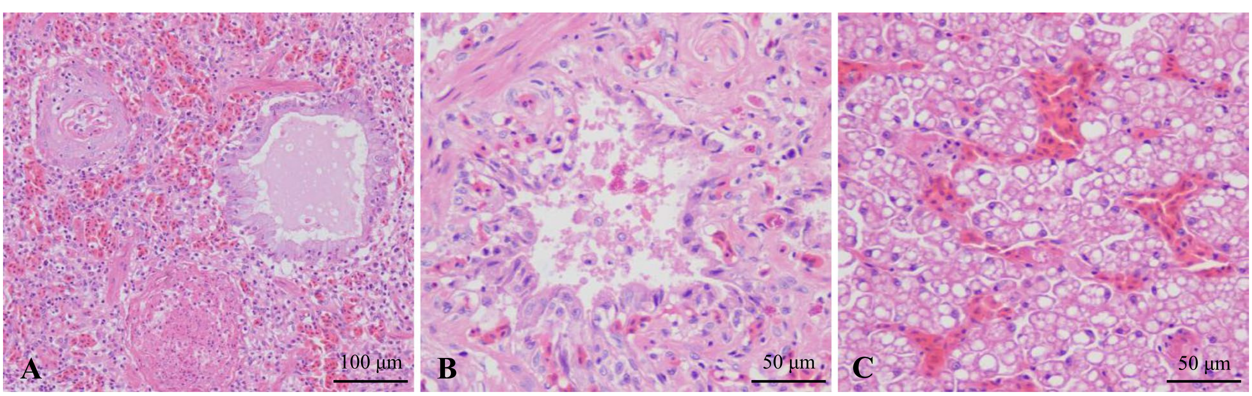

Fig. 3 Histopathological examination. **A** and **B** show infected lung tissue, and **C** shows liver tissue; **A** shows pulmonary hyperemia and fibrosis; **B** shows pulmonary epithelial cells falling off the alveolus; **C** shows hepatocyte steatosis

Treatment

Gentamicin was given through feed or drinking water by the owner before we received the case. The clinical symptoms for one-year-old *Elaphe carinata* were relieved to some extent, but no obvious mortality changed. Two rounds of treatments were given after the lab results. For the first round, amikacin was administered intratracheally at 5 mg/kg twice in 3 d, and

Table 1 Drug sensitivity determination results

Susceptibility paper	Drug sensitivity determination results			
	Staphylococcus sciuri	*Providencia vermicola*	*Salmonella enteritis*	*Vagococcus fluvialis*
Florfenicol	R	S	R	R
Clindamycin	R	R	R	R
Polymyxin B	S	S	S	R
Cephalothin	S	S	R	R
Ofloxacin	S	S	S	S
Streptomycin	R	R	R	S
Gentamicin	I	S	I	S
Doxycycline	R	R	I	R
Cefradine	R	R	R	S
Furazolidone	S	S	S	S
Lincomycin	R	R	R	S
Kitasamycin	R	R	R	R
Erythromycin	R	R	R	R
Norfloxacin	R	R	R	R
Amoxicillin	R	R	R	R
Kanamycin	I	S	I	R
Neomycin	S	S	S	R
Ceftriaxone sodium	I	I	S	S

The isolated pathogens were resistant to most antibiotics and sensitive to cefradine, ceftriaxone sodium and gentamicin

gentamicin was administered intratracheally at 3 mg/kg twice in 4 d. For the second round, amikacin was administered intramuscularly at 5 mg/kg followed by a halved dose for 3 d, and ceftriaxone was administered intramuscularly at 20 mg/kg twice for 4 d. Multivitamins, especially vitamin A, were suggested as additives for snakes as needed.

Outcome and follow-up

Clinical signs for *Elaphe carinata* were under control, and the mortality was close to 0% after treatments. However, treatments for *Ptyas mucosus* seedlings did not work well, and there were two potential causes. The first limitation was the poor administration technique. The owner frequently penetrated from the dorsal to ventral side of the body walls from the injection spot with needles, and seedlings died quickly after whole-body penetration. The second was poor drug dosage control for small body size.

Discussion and conclusions

Seventy-five percent of snake diseases are caused by bacterial infections, and some of them are opportunistic pathogens from normal intestinal flora. Salmonella is one of the normal flora constituents in reptile intestines (Whiley et al. 2017). The diagnosis of Salmonella infection usually requires isolation and identification from infected tissues. Salmonellosis often acts as a secondary infection for immune-suppressed animals. Antibiotic treatments can inhibit its growth but are difficult to sterilize. In the United States, reptiles are responsible for approximately 6% of human salmonellosis cases, and in southwest England, 27.4% of Salmonella cases in children under five years of age are associated with reptile exposure. Multidrug resistance occurs in Salmonella (Murphy and Oshin 2015). Therefore, accurate diagnosis and treatments are needed for salmonellosis.

Providencia spp. can cause urinary tract infection and intestinal diseases in humans. Strains have been isolated from swine heart, liver, spleen and other parenchymal organs of animals that died of sepsis. It has also been isolated from liver, lungs and kidneys of diseased Rhizomyidae, and it can kill mice (Tang et al. 2017). This is the first report of *Providencia vermicola* isolated from reptiles.

Vagococcus fluvialis is a unique gram-positive bacterium that can infect a variety of animals. The first strain was found in 1989. *Vagococcus* is pathogenic to *Oncorhynchus keta* at temperatures below 12°C. It can infect subadults and adults of *Oncorhynchus mykiss*, with mortality rates as high as 20 to 50% (Eldar and Ghittino 1999). Vagococcus mainly causes fibrin deposition and visceral hemorrhage in e heart, spleen and liver. Fish infected with Vagococcus mainly manifest with lethargy, ataxia, damage to the eyeballs, and bleeding from jaw, eye, mouth, abdominal cavity, pelvic fin, anus, furuncles and erosive lesions on the side of the body (Garcia et al. 2015; Michel et al. 1997; Ruiz-Zarzuela et al.2005). This is the first report of *Vagococcus fluvialis* isolated from reptiles.

Staphylococcus sciuri was first reported by Kloos in 1976 (Kloos et al. 1976). With the gradual discovery of various animal infection cases, isolation and identification of bacteria and case analysis have become the main research direction for researchers. Studies have found that *Staphylococcus sciuri* is highly pathogenic. Human beings and a variety of animals can become infected from it. Diseases such as meningitis and urinary tract infections can occur now in humans, dairy cowsand Rhizomyidae (Ojkic et al. 2008; Marek et al.2016; Zhang et al.2016). Researchers have found that the bacteria can infect aquaculture animals, such as *Pelteobagrus fulvidraco*, *Mauremys mutica*, *Monopterus albus*, etc., and even cause death in severe infections. In addition, this is the first report on infection in snakes in China.

In the early stage of this case, the owner had used random antibiotics as treatments, and decreased clinical signs were seen, but symptoms returned, and mortality increased again. Bacterial culture and drug sensitivity tests should still be performed to help choose appropriate drugs before treatment. Gentamicin can be given at 2.5 mg/kg twice in 3 d for snake pneumonia or enteritis before cultures come back.

The way drugs administered is worth mentioning. Reptiles do not need to consume food as frequently as mammals. Adult snakes take 5–7 d for complete digestion after a full meal. As a result, it is not an effective way to give medicine through feeding or drinking water. Individual drug injection is a more appropriate method, although it costs more labor work, and clients usually need training. In our case, clients frequently penetrated snake seedling body walls. When damage is made into the lungs, immediate death can occur. When damage is made into other organs, such as the kidneys, delayed death could occur.

Environmental management and force feeding protocols should be taken into account for sick snakes in addition to treatments. For systemic bacterial infection, the temperature could be raised at least to the upper limit of the optimum temperature, and the old pad should be replaced with a new easy clean pad. Older snakes can be raised on a smooth and clean floor. Water fountains should always be thoroughly cleaned, preferably with standing water.

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Authors' contributions
Conceived and designed the experiments: YS and YX. Provided the material: YS. Performed the experiments: YX, YP and SQ. Analyzed the data and wrote the paper: YX, SL, YP and SQ. Revised the article: YS. The author(s) read and approved the final manuscript.

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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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