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Full Length Research Paper

Studies on hemorrhagic pneumonia in *Moschus sifanicus*

H. Lu[1]*, Y. D. Huang[2], B. Y. Zhao[1], J. J. Hu[2], Q. F. Li[1] and R. W. Dieter[3]

[1]Veterinary Internal Medicine, College of Veterinary Medicine, Northwest A & F University, Yangling, 712100, People's Republic of China.
[2]Veterinary Internal Medicine, College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, 730070, People's Republic of China.
[3]Neurobiology, Institute of Medical Chemistry, DNS, University of Veterinary Medicine, Vienna, A-1210, Austria.

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A series of investigations were carried out including epidemiology, etiology and pathology on hemorrhagic pneumonia in *Moschus sifanicus*, which had prevailed in Xinglong Mountain National Nature Reserve District in Gansu province of China. The results indicated that the prevalence of this disease could be correlated with local humidity in Xinglong Mountain in Gansu province of China. The disease is caused by single infection of *Pasteurella multocida* or mix of *P. multocida*, *Escherichia coli* and *Pseudomonas aeruginosa*, and is a contagious disease. The pathological changes were mainly manifested in the vessel wall of bronchia and bronchiole appeared congested, bleeding, edemic with infiltration of inflammatory cells, mucosa of bronchiole degenerates, with the presence of necrosis and exfoliation, pulmonary alveolus generated suppuration, disaggregation and necrosis. It was concluded that the diseases are mainly caused by local bacteria and affected *M. sifanicus* finally die of hemorrhagic or purulent, necrotic pneumonia.

Key words: *Moschus sifanicus*, hemorrhagic pneumonia, pathologic changes, epidemiology, pathogens, humidity.

INTRODUCTION

Moschus sifanicus are small ruminants that belong to the Cetartiodactyla order of the deer family and musk subfamily. Most of them inhabit China, Burma, Northern India, Northern Vietnam and the Himalayan region (Green, 1986). For a long time, the musk deer has been harvested for the musk secreted by the musk gland in male, which is one of the oldest raw materials used in the perfumery industry because of its fixative and scent properties. China is one of the most important countries known for musk deer distribution (Yang et al., 2002), mainly in Qinghai, Gansu, Xizang, Ningxia, Sichuan and Yunnan Province (Zheng et al., 1979; Sheng, 1992). *M. sifanicus* was listed in the first category of the Chinese State Key Protected Wildlife List in 2002.

In recent years, there had been evidence showing that the population of *M. sifanicus* had a marked decline in Xinglong Mountain National Nature Reserve in Gansu province of China. This mainly resulted from hemorrhagic pneumonia of unknown pathogen and caused considerable economic losses to *M. sifanicus* production (Lu et al., 2008). As a consequence, many veterinary technicians and specialists were organized by the local government to attempt prevention and treatment, but there has been no effective result. At present, prevention and control of the disease are based on the use of antibiotic drugs. However, prevention and treatment of this disease are becoming less effective in *M. sifanicus*, due to increase in resistance. The morbidity and mortality rates were as high as 76.42 and 55.02% in 2003, respectively. So the disease is considered as one of the main constraints in improving the productivity of small

*Corresponding author. E-mail: jjlhml@nwsuaf.edu.cn. Tel: +86-29-87092429. Fax: +86-29-87091032.

Table 1. The annual average local temperature and humidity during 1994 - 2003 (n = 12).

Index	Year									
	1994	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
Temperature(℃)	6.7 ± 0.4	6.6 ± 0.3	5.3 ± 0.3	6.9 ± 0.5	7.5 ± 0.8	7.3±0.6	6.8 ± 0.7	6.7 ± 0.4	7.5 ± 0.9	7.2 ± 0.7
Humidity(º)	65.2 ± 6.6	66.1 ± 7.2	68.0 ± 5.6	67.3 ± 6.4	68.5 ± 8.5	67.5±4.8	69.4 ± 7.6	69.8 ± 4.3	70.6 ± 7.5	71.3 ± 8.2

ruminants in the regions where it is found, for example, the Nature Protection District of Xinglong Mountain in Gansu Province of China.

The aim of this work is to the study the causative agent of the disease that causes high morbidity and mortality in *M. sifanicus*, with a view to offering experimental reference to prevention and treatment of this disease and improving local *M. sifanicus* production.

MATERIALS AND METHODS

Epidemiological investigations and clinical examination

Detailed investigations on the epidemiology of hemorrhagic pneumonia in *M. sifanicus* were carried out in the affected area. These included ascertaining the history, incidence, characteristics and regularity of the diseases, the natural ecological environmental conditions as well as the effects on local animal husbandry. For this study, many local people were interviewed including both the feeder, whose *M. sifanicus* were severely affected by the disease, and the local dwellers living in the area for many years. This was done to get their advice and opinion on the disease and gather background information about the epidemiology. Data on the natural ecological and environmental conditions and on the effects of this disease were obtained from local records and annual reports provided by the local government.

The clinical examination included both visual observations and physical examination. The clinical signs were recorded by direct observation while monitoring *M. sifanicus* in the field, noting in particular manifestations related to hemorrhagic pneumonia as well as appetite and cough was carried out. Selective examinations were carried out in cases of hemorrhagic pneumonia in *M. sifanicus* by routine clinical diagnostic methods, such as auscultation and palpation, percussion and determination of the body temperature.

Etiology and pathogenesis

Affected animals

Twenty (20) affected *M. sifanicus* including 10 females and 10 males, of age between 11 days and 9 years were collected from artificial feeding in Xinglong Mountain National Nature Reserve in Gansu province. All these animals showed obvious signs of hemorrhagic pneumonia, including cough and nasal discharge and augmentation of body temperature. Secretion of trachea and nasal cavity, typical lung, liver and spleen of affected naturally dead *M. sifanicus* were collected using asepsis method.

Healthy controls

The 20 *M. sifanicus*, 10 females and 10 males, selected for the study were 11 days to 9 years in Xinglong Mountain National Nature Reserve of Gansu province, where the diseases had not been reported previously. All these animals were in good health condition based on clinical examination.

Pathological examinations

Routine post-mortem pathological examinations (Zhu and Zhu, 1988) were carried out by both naked-eye examination of the tissues and histological examination.

The preserved tissue samples were embedded in paraffin wax and sectioned with a microtome. The sections were stained by hematoxylin and eosin (H&E) stain and then examined under an optical microscope (Li., 2003).

Etiological examinations

By adopting routine microbiological methods (Yao, 2002), systemic studies were carried out on pathogens of hemorrhagic pneumonia.

Statistical analyses

Differences of the data were assessed by student's *t*-test.

RESULTS

Epidemiology

The affected area is located at 35.38 - 35.56°N latitude and 103.50 -104.10°E longitude, and is situated in the Xinglong Mountain of Yuzhong county in Gansu province at an average elevation of 2,000 - 2,100 m above sea level (the prominent peak 3,670 m, the lowest altitude 1,800 m). The annual average atmospheric temperature of this area is 5.3 - 7.5°C, and the annual average precipitation is 650 - 700 mm (Table 1), rainy season is mainly centred in July, August, September and the frost-free season is 70 - 130 days long. Forage of *M. sifanicus* is mainly composed of leaves and concentrated feed. They were fed twice every day, separately at six o'clock and nineteen o'clock in summer, and eight o'clock and seventeen o'clock in winter. *M. sifanicus* fed fed in pen behaved with a day-start activity climax in

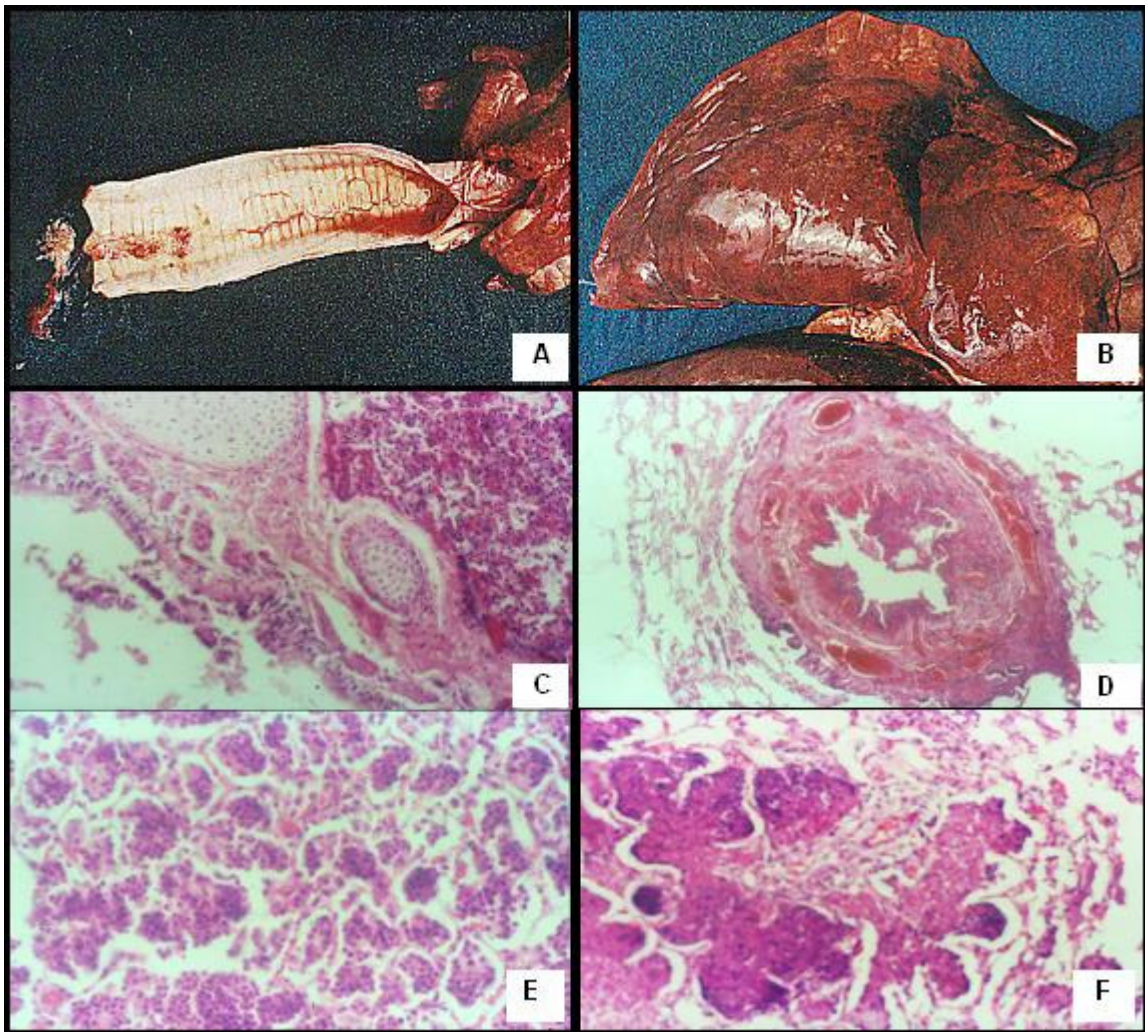

Figure 1. (A) Mucosa of trachea showed severe bleeding and congestion, and secretion largely filled it. (B) Lung appeared to be bleeding, congested with gore and emphysema. (C) Vessel wall appeared congested, epithelium mucosae degenerated, necrosis and exfoliation in bronchia using hematoxylin and eosin (H&E) stain, original magnification was ×100. (D) Vessel wall showed congestion, mucosa degeneration, necrosis and exfoliation in bronchiole using H&E staining, original magnification was ×100. (E) Alveolar space filled erythrocytes, pus cell, leucocyte using H&E staining, original magnification was ×200. (F) Alveolus cavity were full of large inflammation exudation, alveolus appeared crack, putrescence using H&E staining, original magnification was ×100.

summer instead of midday activity climax in winter, and also exhibited a dusk activity climax in summer and autumn. Furthermore, a midnight activity climax was highly consistent in these seasons in *M. sifanicus*

Clinical signs

In the initial stages of this disease, the affected animals manifested short and painful cough, anorexia, tachypnea, pyrexia (40.94 ~ 41.94℃). Subsequently, they showed a serious or purulent nasal discharge secretion, endophthalm, facial tumefaction, endless cough and continuous phlegm stridulous sound. In the final stages, severe cases showed complete anorexia, an unwillingness to get up, dyspnea with weak cough and coarse phlegm rale. These cases commonly evolved to trance before they died of heart failure due to dyspnea.

Pathology

Autopsy findings

It was obvious that mucous membrane of larynx and trachea were found congested, bleeding, gore and bleeding point to some degree. Grume and purulent secretion were full of trachea (Figure 1A). The lung appeared congested, hemorrhage, scraggly surface, paleness, bulk augment, texture rigidity changed as liver

and meat appearance, slice side wetness; mucosa of bronchia manifested congest and tumefaction (Figure 1B).

Histopathology

Epithelium mucosae of bronchia showed necrosis and exfoliation. There were largely inflammatory and necrotic substance in bronchia cavity, the vessel wall of bronchia showed congestion, bleeding, edema and infiltration of inflammatory cells, mucosa of bronchiole degeneration, necrosis and exfoliation, the vessel wall of bronchiole appeared congested and bleeding; there were largely putrescence substance and neutrophilic granulocyte, pyocyte, erythrocyte, epithelial cell and mucus in the bronchiole cavity (Figures 1C and 1D). Abundant erythrocytes, pus cell, leucocyte were assembled and inflammatory exudate could be observed in alveolar space, pulmonary alveolus generated suppuration, disaggregation and necrosis; these then formed abscess and necrosis focus (Figures 1E and1F).

Etiology

Bacteria test

Three bacteria species were isolated from affected animals. The colonial morphology, staining and culture characteristics were as follows: One of the bacteria specie which (1#) appeared gram-negative, was either ball or short rod with obvious of two poles coloration and it had retarded growth on MacConkey agar medium. The second also appeared (2#) gram-negative and showed dispersive distribution or gemination array, had major, face side lubricity, fringe trimness, pink or red colony on MacConkey agar medium and manifested black and metal-gleamy microcolony on eosin methylene blue agar medium. The third (4#) also appeared gram-negative, simple or gemination range and manifested macro-lubricity, fringe evenness, centre raised and prasinous colony.

Virus test

The agglutination phenomenon did not find red cells of chick, pig and rabbit in the hemagglutination test. Centrifugation strip and chick embryo allantoic fluid and cell culture medium were observed by electron microscope, but virus granule was not found among them.

Epiphytic test

Pathological change in tissue was evaluated by inoculating in TTC-Sabouraud's agar plate and culturing for 24 - 48 h at 35℃. However, there was no growth of colony in the plate.

Serological test

Agglutination reaction could be observed between 1#, 2# and 4# bacteria and serum of affected *M. sifanicus.* Precipitation strip were not found in agar gel diffusion test.

According to colonial morphology, staining and culture characteristics, biochemical test (Table 3) and serological test of isolated bacteria, it could be ascertained that 1# bacterium was *Pasteurella multocida*, 2# was *Escherichia coli* and 4# was *Pseudomonas aeruginosa*.

DISCUSSION

The results of the preliminary epidemiological and pathogenic bacteria investigation indicated that hemorrhagic pneumonia of *M. sifanicus* was caused by local resident bacteria in Xinglong Mountain in Gansu province of China.

The incidence of hemorrhagic pneumonia was correlated with temperature and humidity, especially local humidity which was primarily one of the inductive factors on this disease (Lu et al., 2004). According to data analysis (Tables 1 and 2), lower correlation had no significant differences between the morbidity of hemorrhagic pneumonia and the humidity from 1994 to 2003 ($R = 0.31$, $P>0.05$). Unexpectedly, the incidence of hemorrhagic pneumonia was significantly positively correlated with humidity during 1994 - 1999 ($R = 0.89$, $P<0.05$); it actually showed the most significantly positive correlation during 2000 - 2003 ($R = 0.99$, $P<0.01$). This could be related with the fact that environment and condition of breeding were changed after 2000. However, this result suggests that the local humidity was the most important inductive factor on this disease. Although the data did not show that the local temperature had any obvious influence on this disease, it might be correlated to population of *M. sifanicus* which increased gradually every year, and veterinarians and feeders had frequently been replaced.

Pathogenic microorganism enter lung by respiratory tract, primarily through bronchioles, quickly spread to peripheral stroma, and beget serous or fibrinous inflammation. Subsequently, it affects the whole lung through the lymphatic and blood vessels. Pathogenic microorganism gets into the bronchus and affix close to cilia and epithelail cell membrane so that they are not discharged by mechanism of grume-cilia. They clung to epithelium release product of metabolism which poisons cilia and cell membrane leading to cilia exfoliation and cell membrane lesion. Furthermore, pathogenic microorganism invade organism, which bring complicated

immunological pathogenesis and produce extensive immune abnormity reaction and injury to the immune

Table 2. The annual morbidity, mortality and cure rates of diseases in *Moschus sifanicus* during 1994 - 2003.

Years	All diseases[b]			Hemorrhagic pneumonia		
	Morbidity (%)	Mortality (%)	Cure rates (%)	Morbidity (%)	Mortality (%)	Cure rates (%)
1994 (31)[a]	32.26	22.58	30.00	19.35	16.13	16.69
1995 (59)	38.98	30.51	21.24	27.12	23.74	12.50
1996 (76)	46.05	38.14	17.14	31.58	28.95	9.09
1997 (89)	44.94	32.58	27.50	30.34	26.92	17.24
1998 (141)	43.26	31.20	27.87	31.91	28.37	11.11
1999 (184)	41.85	29.34	29.85	28.26	22.28	21.15
2000 (195)	53.33	38.46	27.88	49.23	31.79	29.71
2001 (217)	69.12	50.69	26.67	57.60	38.71	32.80
2002 (240)	77.92	59.17	24.06	67.92	47.50	30.39
2003 (229)	80.43	64.19	19.02	76.42	55.02	24.03

[a]Number of animals in parentheses; [b]Bronchitis, gastroenteritis, urolithiasis, thermoplegia and stress disease.

Table 3. The result of biochemistry tests of pathogenic bacteria.

Bacterium biochemical reagent	*P. multocida*	*E. coli*	*P. aeruginosa*
glucose	⊕/+	⊕	+
fructose	⊕	⊕	+
sucrose	⊕	⊕	-
mannose	⊕/ -	⊕	-
galactose	⊕	⊕	-
mannitol	+	+	+
xylose	+	+	-
lactose	-	+	-
inositol	-	-	-
oxidase	+	-	+
MR test	-	+	-
VP test	-	-	-
Gelatinliquefaction test	-	-	+
Indole test	+ / -	+	-
Hydrogen sulfide test	+	-	-

⊕deposit acid and aerogenesis, **+** deposit acid/positive, **-** unchanged/negative

system.

Through histopathological observation of dead *M. sifanicus*, it was confirmed that *P. multocida*, *E. coli* and *P. aeruginosa* were primarily pathogens for this hemorrhagic pneumonia using bacteriologic and serological test. Hemorrhagic pneumonia main pathologic changes were hemorrhagic or fibrinous suppurative pneumonia in *M. sifanicus*. 3# is gram positive, ranging in thyrsiform, it manifested humidification, lubricity, tortulose, offwhite and circular pattern colony in ordinary cultural medium, it grows in blood agar plate, but with nonoccurrence hemolysis; 18 h broth culture of this bacteria was injected to small white rat and they all survived over 7days, it showed that the bacteria were not pathogenic through bacterial virulence test, therefore it was not mentioned in biochemical and drug susceptibility test.

In the animal treatment, sheep rather than *M. sifanicus* were selected as experimental animal, mainly due to cost. Moreover, their population had gradually decreased due to activities of their natural enemy and some diseases. In addition, sheep and *M. sifanicus* are both small ruminants with great similarities between them. We had successfully replicated pathological pattern, which was consistent with clinical signs and pathological changes of hemorrhagic pneumonia of *M. sifanicus* (Lu et al., 2008).

Hemorrhagic pneumonia of *M. sifanicus*, which had recently prevailed in Xinglong Mountain National Nature Reserve in Gansu province, was an ordinary infectious

disease. Due to the fact that the causative pathogen has not been identified, prevention and treatment of the disease had showed no effect, leading to tremendous economic loss. Some researches of *M. sifanicus* had been principally fastened on these domains, which has geographic differentiation (Xia et al., 2004) ecology (Zheng and Pi, 1979; Liu et al., 2001). In conclusion, from the result of the present study, important experiment reference has been offered for the development and clinical application of triple combined inactivated vaccine of *M. sifanicus*. This will help provide a positive and profound protection and utilization of costly wild animals that were close to being extinct.

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