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The Characteristics of New Zealand Rabbit Model for Infection with Pathogens of Fungal Skin Disease in Asian Elephants in Chongqing Zoo

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Abstract: Asian elephants in Chongqing zoo were repeatedly affected by fungal skin disease recent years. This study sought to establish New Zealand rabbit models infected with *Microsporum canis* which was pathogens of fungal skin disease developed in Asian elephants in Chongqing zoo. Bacterial colonies were incubated in Sabouraud dextrose agar at 28°C for 14 days and then prepared into bacterial suspension and inoculated on the dorsal skin of the rabbits. After 10 days inoculation, the affected skin of rabbits developed advent clinical symptoms such as scab and desquamation that were extremely similar to those of fungal skin disease in Asian elephants. The skin tissue at a size of 1×1 cm was collected. The paraffin section was prepared and stained for argentaffin. The pathological section showed that brown orange *Microsporum canis* was visible in skin tissue after argentaffin staining. The results showed that pathological New Zealand rabbit model for infection with pathogens of fungal skin disease developed in Asian elephants in Chongqing zoo was successfully established.

Key words: Animal models, infection, *Microsporum canis*, symptoms, pathogenes

INTRODUCTION

From 2004 to 2009, Asian elephants in Chongqing zoo were repeatedly affected by fungal skin disease (Wei *et al*., 2012). The affected skin showed distinct degrees of white spot, desquamation, crack, pruritus and scab. Skin tissues of affected elephants were collected and incubated in Sabouraud dextrose agar for the formation of bacterial colonies like gray white hyphae ribbons. Two common fungus primers, ITS1 and ITS4 were used for PCR amplification and sequencing of isolated and cultured fungi. The results showed that the fungus was 99.7% homologous to *Microsporum canis* (Yang *et al*., 2010) demonstrating that fungal skin diseases in Asian elephants were caused by *Microsporum canis* infection.

Microspormn canis is a common pathogenic fungus (Wang, 2012) parasitic in the keratins of the animal's clothing hair, epidermis and claws which can be transmitted between human beings and animals. In this study, the animal model was prepared by infecting New Zealand rabbits with *Microspormn canis* isolated from the affected parts of Asian elephants to further study the skin diseases caused by *Microspormn canis*. The results showed that New Zealand rabbits affected with *Microspormn canis* had clinical symptoms quite similar to those of Asian elephants infected with the same bacteria. The results of pathological section showed that *Microsporum canis* was present in skin tissue of affected rabbits. The pathological New Zealand rabbit model for infection with *Microsporum canis* in this study is of significance for the study of treatment of skin diseases caused by *Microsporum canis* infection.

MATERIALS AND METHODS

Experimental animals: The 20 New Zealand rabbits (10 males and 10 females) of clean grade at a weight of 2.0±0.1 kg were provided by the Experimental Animal Center of Chongqing Medical University.

Bacterial strain and reagents: *Microsporum canis* strain was isolated from affected skin of Asian elephants with fungal skin diseases in Chongqing zoo. The Sabouraud dextrose agar was purchased from Qingdao High-tech Park Haibo Biological Technology Co., Ltd. The wood lamp was purchased from Southwest Animal Medical Equipment Center. The silver nitrate was purchased from Qingdao Jacob Chemical Reagent Sales Co., Ltd. The anhydrous sodium acetate was purchased from

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Tianjin Zhiyuan Chemical Reagent Co., Ltd. The 1,4-hydroquinone was purchased from Tianjin Yongda Chemical Reagent Co., Ltd. The anhydrous sodium sulfite was purchased from Shanghai Aibi Chemistry Preparation Co., Ltd. The xylene was purchased from Chongqing Chuandong Chemical Co., Ltd. The neutral gum was purchased from Shanghai Zhanyun Chemical Co., Ltd.

Inoculation of bacterial suspension onto New Zealand rabbits

Preparation of bacterial suspension: The bacterial colonies incubated in Sabouraud dextrose agar at 28°C for 14 days were scraped and ground. After addition of sterile normal saline, the absorbance of the bacterial suspension was adjusted to 1.0 at 550 nm. The dorsal clothing hair on both sides of the rabbits was shaved and a skin area as size of 2×2 cm was exposed. The area was rubbed with sandpaper until oozing of blood but not bleeding. The 2 mL of prepared bacterial suspension was pipetted and inoculated on the wounded area caused by sanding and coated evenly. The animals in the control group were coated with the same amount of normal saline. And then the animal's mental state, appetite and symptoms of inoculated areas were observed every day. The wood lamp was used only for adjuvant diagnosis.

Identification of fungi: Skin scales of the affected rabbits were collected and placed in a sterile tube with an appropriate amount of sterile normal saline. The tube was shaken at 200 rpm for 1 h. And then 0.2 mL of the solution was pipetted and added to Sabouraud Dextrose Agar, cultured at a constant temperature of 28°C. The fungi were investigated by microscopic examination using microscope.

Pathological section of skin tissues: When New Zealand rabbits infected with *Microsporum canis* developed typical clinical symptoms on the affected area, the full-thickness skin tissue at a size of 1×1 cm was extracted from the affected area of the rabbits. The paraffin section was prepared (the thickness of sections was 4 μm). Argentaffin staining and hematoxylin staining were carried out.

Argentaffin staining

Preparation of silver nitrate buffer: Add 5 mL of 1% silver nitrate solution and 10 mL of 0.2 mol L^{-1} acetic acid buffer which is under a pH of 5.6-85 mL distilled water.

Preparation of reducing solution: Add 1 g of 1,4-hydroquinone and 2.5 g of anhydrous sodium sulfite into 100 mL of distilled water. The above two solutions should be freshly prepared before use.

The operation procedures of argentaffin staining: The section was deleted, washed with distilled water, placed in pre-heated silver nitrate buffer at 60°C for 3 h and then immediately washed with distilled water, added to reducing solution and reacted at 45°C for 5-10 min, washed with distilled water and observed under microscope. And then the section was dehydrated using 95% absolute ethyl alcohol, turned to transparent by xylene and blocked with gum.

Hematoxylin staining (preparation of hematoxylin staining solution): The 2000 mL of distilled water and 80 g of aluminium potassium sulfate were heated to dissolve. Then add 200 mL of anhydrous ethanol and 10 g of hematoxylin. The hematoxylin solution dissolved in anhydrous ethanol was slowly injected into 2000 mL of aluminium potassium sulfate solution, brought to boiling. The 3 g of mercuric oxide and 20 mL distilled waters were added to hematoxylin solution and stirred to obtain dark blue hematoxylin staining solution. The solution was kept under protection from light. Each 100 mL of hematoxylin solution was added to 2-3 mL of ice acetic acid and mixed thoroughly immediately before utilize.

Procedures of hematoxylin staining: Hematoxylin staining was maintained for 5-15 min, washed with water, waited for a moment for color separation with 0.5-1% hydrochloric acid alcohol (prepared with 70% alcohol) rinsed with running water for 15-30 min, washed with distilled water and observed under microscope. And then the section was dehydrated by 95% absolute ethyl alcohol, turned to transparent by xylene and blocked with gum.

RESULTS AND DISCUSSION

Pathological changes of the skin surface: After 6 days inoculation of *Microsporum canis*, the New Zealand rabbits started to develop clinical symptoms on the affected skin. After 14 days inoculation, the symptoms became more advent. The affected skin became stiff, formed scab and had desquamation. After exposure to the wood light, the affected area infected with *Microsporum canis* emitted green fluorescence while no infection occurred in the healthy control group. During study, rabbits had a normal appetite and mental state. Compared to the conditions of desquamation, crack and scab on the affected skin of Asian elephants, it could be found that the clinical symptoms of both were extremely similar (Fig. 1-3).

Identification of fungi: After inoculation of *Microsporum canis*, the rabbits developed typical

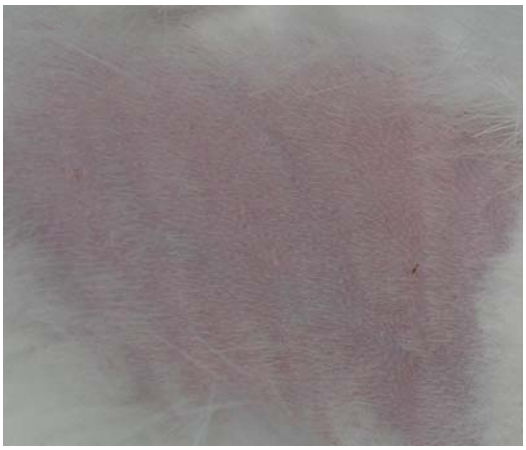

Fig. 1: The New Zealand rabbit skin symptom of the control group

Fig. 2: Skin symptom of New Zealand rabbit infected with *Microspormn canis*

Fig. 3: Skin symptom of Asian elephant with skin disease caused by fungal

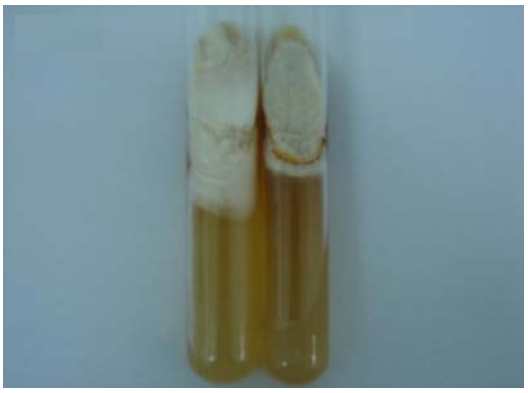

Fig. 4: Microscopic examination of *Microspormn canis* isolated from New Zealand rabbit (1000x)

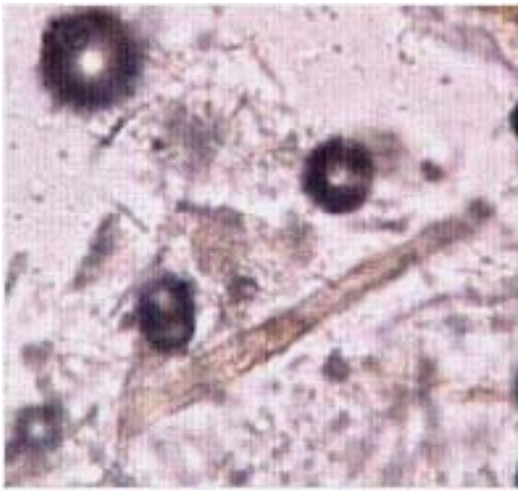

Fig. 5: Morphology of *Microspormn canis* colony isolated from New Zealand rabbit

pathological symptoms on the inoculated skin. Skin scales of the affected area were scraped for microscopic examination. The results showed that round microsporum was visible under the microscope. The scales of affected area were incubated in Sabouraud Dextrose Agar for 14 days and typical *Microsporum canis* was present (Fig. 4 and 5).

Pathological changes of skin tissue section: When clinical symptoms occurred on inoculated skin of rabbits, the skin tissues were extracted and paraffin section was ready. Argentaffin staining and hematoxylin staining were carried out. In the control group, only hair follicle gland in the epidermis became brown orange and not round *Microsporum canis* was visible after argentaffin staining. But in the experimental group, *Microsporum canis* was detectable in the dermis and became brown and round due to argentaffin staining (Fig. 6-9).

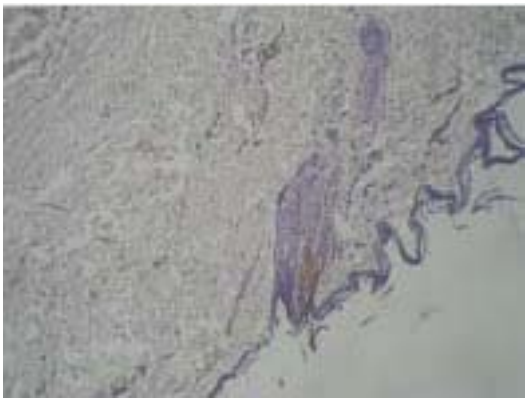

Fig. 6: Skin biopsy of the control group stained with silver (100x)

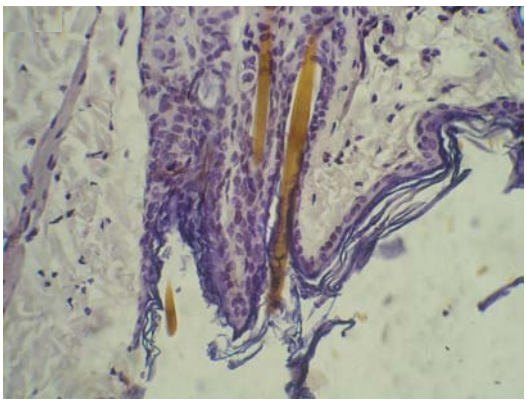

Fig. 8: Skin biopsy of the control group stained with silver (400x)

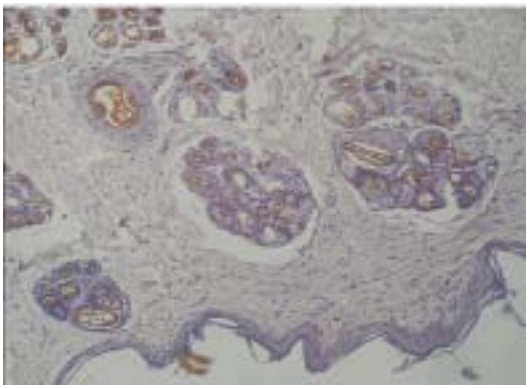

Fig. 7: Skin biopsy of the treatment group stained with silver (100x)

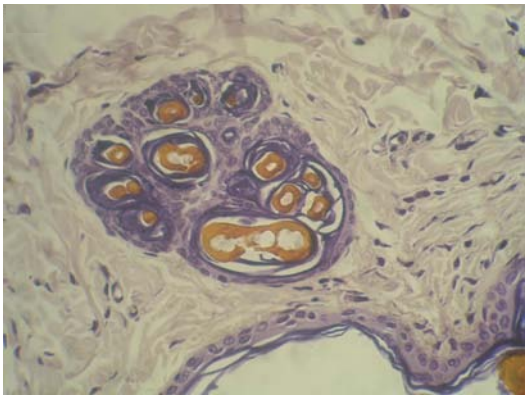

Fig. 9: Skin biopsy of the treatment group stained with silver (400x)

Microsporum canis is a common pathogenic fungus and its clinical manifestations are skin papule, scab, desquamation, pruritus and vague edge. Reports showed that the morbidity of fungal disease caused by *Microsporum canis* ranked as one of the highest four among superficial fungal diseases (Hu, 2005). In recent years, Asian elephants in Chongqing zoo developed fungal skin disease successively. The cause was found to be *Microspormn canis* after clinical diagnosis, isolation and incubation of affected materials and molecular biological identification. In this study, the animal model was prepared by infecting New Zealand rabbits with *Microspormn canis* isolated from the affected parts of Asian elephants in order to study the treatment of *Microspormn canis*.

CONCLUSION

Results showed that the New Zealand rabbits infected with such bacteria improved clinical symptoms similar to those in Asian elephants. Since, Asian elephants are not investigational animals, it is impossible to collect pathological tissue samples from them. Thereby, this study collected skin tissues from affected areas of New Zealand rabbits and prepared pathological sections for argentaffin staining and well observed the infection conditions of *Microspormn canis* in skin tissues. In this study, New Zealand rabbits were selected for establishing the model for infection with *Microspormn canis* since, rabbits are sensitive to skin stimulation and have responses similar to human beings and they are often used for study of local skin reactions caused by toxics, drugs and cosmetics. The pathological sections were performed for argentaffin staining (Gong and Zhan, 1993) and brown orange round *Microsporum canis* was observed in the dermis due to argentaffin staining which demonstrated that the pathological New Zealand rabbit model for *Microsporum canis* infection with isolated pathogens of disease developed in Asian elephants in Chongqing zoo was successfully established and it is of significance for the study of treatment of skin diseases caused by *Microsporum canis* infection.

ACKNOWLEDGEMENTS

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