Review

Endangered Zoonotic Fungal Species from Chicken (*Gallus gallus domestics*)

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ABSTRACT

Lophophyton gallinae (*Microsporum gallinae*) is a zoophilic fungus that causes ringworm in chickens and related species, and occasionally in humans. There are 45 human cases worldwide including a Japanese case from Okinawa in 2009. After the finding of the human *L. gallinae* case, 793 chickens in Japan, including 293 from the mainland and 500 from the Nansei Island areas, were investigated to determine the prevalence of dermatophytes and their related fungal species. The survey was carried out from December 2008 to March 2013. Various dermatophytes and related fungal species were isolated from the studied chickens, with a prevalence of 24.6%. In total, 224 dermatophytes and related species were isolated in the survey. The most commonly isolated species included, in descending order of frequency, *Arthroderma multifidum*, *Aphanoascus terreus*, and *Chrysosporium* spp. *Ar. multifidum* and *Ap. terreus* have no record of pathogenicity, and the present isolates of *Chrysosporium* spp. were not matched to pathogenic *Chrysosporium* spp. based on the ITS rDNA sequences. Interestingly, an *L. gallinae* isolate was detected in a male 10-month-old shamo (fighting cock) from the main island. Furthermore, one strain of *Arthroderma simii* was also isolated as the second record in Japan following that from an imported chimpanzee. Although *L. gallinae* and *Ar. simii* are likely to be endemic in our country, the transmission of dermatophytosis from chickens to humans is unlikely to occur because of the reduced chances for citizens to come in contact with chickens due to various factors.

Key words : *Arthroderma simii*, *Gallus gallus domesticus*, *Lophophyton gallinae*, *Microsporum gallinae*, zoonotic fungus

Introduction

*Lophophyton gallinae*¹⁾ (previously known as *Microsporum gallinae*) from chickens²⁾ is a minor zoonotic fungal pathogen following the well-known major ones, such as *Microsporum canis*, which is transmissible mainly from cats, *Trichophyton verrucosum* from cows, and *Trichophyton mentagrophytes* complex from rodents. Interestingly, *L. gallinae* infection in humans are rare. The reports in humans are only 45 cases at the present including from Japan: a case of tinea corporis in subtropical Okinawa in 2009³⁾. Based on the circumstances described below, *L. gallinae* infection in Japan will be most likely be eradicated in the near future. This report describes the current status of zoonotic fungal infections caused by *L. gallinae* and its ecology in Japan.

Humans and chickens

As common knowledge, our ancestors initially tamed chickens for cockfighting. In Japan, cockfighting had been popular since the Heian era. Until the mid-1970s, chickens were kept as companion animals in many households and reared at educational facilities as part of the emotional education program. However, owing to the change in housing conditions and associated difficulty in securing rearing space, noise issues, and other reasons, the number of captive chickens rapidly declined. The number of chickens reared at educational facilities, including kindergartens and elementary schools, is also declining due to concern over avian zoonoses such as influenza and severe acute respiratory syndrome⁴⁾. Cockfighting using the shamo breed (Fig. 1) has also been banned in many areas because of its gambling nature and was completely discontinued after the Great East Japan Earthquake in the area where it had been a part of cultural history.

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Fig. 1. A shamo cock reared in Okinawa.

Epidemiology of *Lophophyton gallinae*

L. gallinae is listed as one of the causative agents for zoonotic dermatophytoses and is distributed worldwide, including temperate and subtropical regions, such as India, Iran, Pakistan, Nigeria, America, Australia, Brazil, and Japan, as well as cold regions, including Sweden, Germany, and Czechoslovakia^{3, 5-16}.

The first case of *L. gallinae* infection in Japan³⁾

The patient was a 96-year-old, otherwise healthy Japanese man, who had been working as a breeder of fighting cocks for more than 70 years. He was bitten on his right forearm by one of the fighting cocks. A few weeks later, two erythematous macules appeared on his right forearm. The macules gradually increased in size and were accompanied by a slight itchy sensation, so he visited our hospital. Clinical examination at the first visit revealed scaled and annular erythematous macules approximately 2.0 cm in diameter in two places on the right forearm. Direct microscopic examination of KOH-prepared lesional scale specimens revealed many hyphal components. He was diagnosed with tinea corporis, treated with a terbinafine cream, and was cured in 1 month.

Incubation of skin scrapings from his skin lesion on Sabouraud dextrose agar yielded white, fluffy, wrinkled colonies producing pink pigment. Septate hyphae and macroconidia with 2–10 septa were observed under light microscopy. The fighting cock responsible for the infection, however, was already culled at his home and could not be examined. Fungal culture of residual feather, scales of the other fighting cocks, and soil in his house did not yield *L. gallinae*.

The prevalence ratio of dermatophytes and their related fungal species in chickens and fighting cocks (Shamo) in Japan

1. Materials and methods

After the onset of *L. gallinae* infection in Okinawa, 793 chickens reared privately and at rearing facilities in Japan, including 293 from the mainland and 500 from the Nansei Island areas, were investigated. Prefectures are not mentioned to comply with the privacy policy. The survey was carried out from December 2008 to March 2013. Approval was obtained from the ethics committee for animal welfare of the University of the Ryukyus (No. 2011-5271 and 2012-5437). Combs from the 793 birds (645 chickens and 148 fighting cocks (Shamo breed)) were examined to determine the prevalence of dermatophytes and their related fungal species. Five-hundred of the chickens were reared by shamo lovers or at rearing facilities in Okinawa (464) and Kagoshima (36), and 293 were from the main island of Japan, mostly from the Kanto region, or the Tokyo metropolitan area^{16, 17)}. The details regarding the chickens are given in Table 1.

Transparent plastic tape 18 mm in width (Scotch Sumitomo 3A, Tokyo, Japan) was cut into strips approximately 10 cm in length. Both 1 cm ends were folded for handling. The adhesive surface (approximately 6 cm in length) was placed on the comb and gently rubbed with both the thumb and index finger to collect scales. The tape was incubated at 42°C for 4 h to eliminate mites. The tape was then stamped on BBLTM MycoselTM Agar (Becton, Dickinson and Company (BD), Sparks, MD, USA) plates in duplicate and cultured at 35°C for up to 28 days.

White- to slightly beige-colored, cottony or powdery colonies, which are characteristics of dermatophytes and related species, were picked and transferred onto potato dextrose agar (DifcoTM Potato Dextrose Agar; PDA, BD) slants and incubated at 25°C for further molecular biological analysis. In addition, we placed a transparent tape on the first colonies that appeared and observed them by light microscopy after staining with lactophenol cotton blue, from which we detected round or pyriform conidia attached at a right angle to the hyaline septate hyphae. Following molecular biological identification, multiple fungal growths from one comb sample found to be different genotypes of identical species were treated as individual isolates^{16, 17)}.

2. Results

L. gallinae was detected from a male 10-month-old shamo from the main island (Figs. 2a-c). The other 10 chickens reared at the same facility were negative for *L. gallinae*. The sequence of the ITS region was 99% homologous to the known *L. gallinae* sequence (DDBJ accession No. AB667976). The strain isolated from the shamo had one-

Table 1. Number of positive samples

Area	Total	Chicken		Shamo (Fighting Cock)	
		Female	Male	Female	male
Mainland	89/293 (30.4 %)	28/115 (24.3 %)	28/113 (24.8 %)	14/21 (66.6 %)	19/44 (43.2 %)
Nansei Islands	106/500 (21.2 %)	46/321 (14.3 %)	40/96 (41.7 %)	4/16 (25.0 %)	16/67 (23.9 %)
Total	195/793 (24.6 %)	74/436 (17.0 %)	68/209 (32.5 %)	18/37 (48.6 %)	35/111 (31.5 %)

Fig. 2. The shamo cock from which *L. gallinae* was isolated.

- (a) Desquamation of the comb can be observed.
 (b) Potato dextrose agar plate, 28°C, 28 days. Note the production of characteristic pink pigment.
 (c) Slide culture, potato dextrose agar, 25°C, 21 days. Formation of claviform macroconidia and oval, pear-like microconidia characteristic of *L. gallinae* was observed.

Fig. 3. Cultural characteristics of *Arthroderma simii* isolate.

- (a) Sabouraud dextrose agar, 25°C, 14 days.
 (b) Takashio agar, 25°C, 14 days.
 (c) Slide culture, potato dextrose agar, 25°C, 14 days.

nucleotide difference compared with the strain isolated from a man in Okinawa (DDBJ accession No. AB560660), which suggests that these strains were not related with each other. The owner reported no special prior history of contact with the shamo strain¹⁶⁾.

Another noteworthy result was the isolation of *Trichophyton simii*, although only one strain (Figs. 3a and b). Chained chlamydospores were produced on potato dextrose agar, while pear-like microconidia similar to those of *Arthroderma benhamiae* isolates were detected on Sabouraud dextrose agar

plate (Fig. 3c). The isolate was the second strain of *T. simii* recorded in Japan following the isolate from an imported chimpanzee in the 1960s¹⁸⁾. Although human cases of *T. simii* infection are also rare, caution is still needed as cases of tinea capitis caused by this species have been reported from Iran and Saudi Arabia.

We isolated various dermatophytes and related fungal species from the studied chickens, with a prevalence of 24.6 %¹⁷⁾. In total, 224 dermatophytes and related species were isolated in this survey. The most commonly isolated species

Table 2. Number of positive samples and details of isolates classified based on areas

	Mainland	Nansei Islands	Total
Number of positive samples (%)	89/293 (30.4 %)	106/500 (21.2 %)	195/793 (24.6 %)
Number of isolates and details	93	131	224
<i>Arthroderma multifidum</i>	17	84	101
<i>Aphanoascus terreus</i>	53	30	83
<i>Chrysosporium</i> sp.	17	11	28
<i>Uncinocarpus queenslandicum</i>	5	0	5
<i>Uncinocarpus reesii</i>	0	2	2
<i>Aphanoascus pinarensis</i>	0	1	1
<i>Amauroascus kuehnii</i>	0	1	1
<i>Trichophyton simii</i>	0	1	1
<i>Gymnoascus petalosporus</i>	0	1	1
<i>Lophophyton gallinae</i>	1	0	1

included, in descending order of frequency, *Arthroderma multifidum*, *Aphanoascus terreus*, and *Chrysosporium* spp. (Table 2).

Ar. multifidum was frequently isolated from privately owned chickens, the reason for which is unknown, but no evidence of infection in humans and animals was found. *Ap. terreus* is a keratinophilic fungus and has been isolated from soil and birds; however, this fungal species has no record of pathogenicity. Identification of *Chrysosporium* spp. isolates at the species level requires further investigation. The fungal species are known causative agents of mycotic disease in poultry and are often isolated from feathers and animal-related environments. Human cases of onychomycosis and systemic infection in a patient with acute lymphocytic leukemia caused by *Chrysosporium* sp. have been reported. However, the present isolates were not matched to pathogenic *Chrysosporium* spp. based on the ITS rDNA sequences¹⁷⁾.

Conclusion

The first Japanese case of *L. gallinae* infection was detected in Okinawa, and the same species was isolated from a shamo cock in the main island of Japan in this study. Moreover, *T. simii* was also detected from a male shamo cock in Okinawa. Although these two fungal species are likely to be endemic in Japan, the transmission of dermatophytosis from chickens to humans is unlikely to occur in Japan, where the chance of human contact with chickens has decreased due to various social factors. However, it is still important to note that causative species of dermatophytosis that are transmissible from chickens exist in Japan.

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Conflicts of interest

None.

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