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Case Report

OUTBREAK OF WHITE PIEDRA IN A FLOCK OF EXTREMEÑA BLUE HENS

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Abstract

This paper describes for the first time a case of avian ringworm, caused by *Cutaneotrichosporon jirovecii* in a small flock of Extremeña blue hens and highlights that the disease could occur in outbreak proportions, with 46.70% of cocks affected. Infected birds had white crusts on the comb, and most affected animals showed white or light brown nodules that were loosely attached to the tip of feathers, but no systemic disease signs were observed. The diagnosis of *Trichosporon jirovecii* was made by morphological identification, and it was confirmed by sequencing amplification product of the PCR using universal primers of the internal transcribed spacer region.

Keywords: outbreak, ringworm, rooster, superficial lesion, *Trichosporon jirovecii*, zoonosis

CASE PRESENTATION

Dermatophytes are one of the most common causes of dermatological problems in poultry. Dermatophytosis is not considered a particularly important disease of intensive poultry, although it can be a problem in some free-range and backyard flocks. Mostly, these diseases occur sporadically, but at times they can occur in outbreak form. *Microsporum gallinae* is the major dermatophyte of gallinaceous species. *Trichophyton simii* and *Microsporum gypseum* have also been described as causative agents of favus in poultry

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(Hubalek, 2000; Kannan et al., 2006), but to the best of the authors' knowledge, no record of *Trichosporon jirovecii* has been found.

Trichosporon is a genus of fungus that appears as yeast-like anamorphic cells and belongs to the order *Tremellales* in the class *Tremellomycetes* (division Basidiomycota). The genus contains a total of 50 species, 17 of which have been reported as pathogens. Among them is *T. jirovecii*. They are widely distributed pathogens inhabiting soil, decomposing wood, air, rivers, lakes, and seawater and infect both invertebrates (ants, beetles, etc) and vertebrates (cattle, sheep, dolphins, etc) including humans (Galligan et al., 2019; Garg et al., 2018; Kotwal et al., 2018; Milan et al., 2018). These fungi are also present in birds' and mammals' faeces (Colombo et al., 2011).

Trichosporon has recently been reclassified based on phylogenetic analyses using sequences from several genes. This reclassification relocated the medically relevant species into three genera: *Cutaneotrichosporon (Cutaneotrichosporon cutaneum, Cutaneotrichosporon jirovecii, Cutaneotrichosporon dermatis, Cutaneotrichosporon mucoides); Trichosporon (Trichosporon asahii, Trichosporon asteroides, Trichosporon coremiiforme, Trichosporon faecale, Trichosporon inkin, Trichosporon japonicum, Trichosporon lactis, Trichosporon ovoides and Trichosporon dohaense)* and; *Apiotrichum* (*Apiotrichum domesticum, Apiotrichum montevideense and Apiotrichum loubieri*) (Liu et al., 2015).

In this study, the affected birds belonged to an Extremeña blue hen farm in southwestern Spain. The Gallina Extremeña Azul (extremenian blue hen) is an ancient breed with dual aptitude that was created in the 1980s. It is currently recognized as a Native Breed in Danger of Extinction. It has smooth feathers, a simple crest, and naked tarsi. It has three varieties of plumage color: blue, dirty white, and black. The plumage is not very tight with rounded feathers. It is a medium-sized bird and the weight of the adult rooster is 3 to 4 Kg and the weight of the hen is 2 to 3 Kg.

The flocks comprised 160 hens and 50 cocks. Male and female chicks were usually reared together until about 17-18 weeks of age when the hens were housed individually in battery cages which were placed about 1 to 2 meters above the ground on leg supports. Tap water and commercial feed were supplied by PVC water pipes and steel feeder troughs outside the cages. The cages are designed so the eggs will roll out of the cage to a holding area using a slanted wire floor.

The males were also housed in individual battery cages where water and feed were offered *ab-libitum* in a separate room, with no physical contact between hens and cocks. The indoor air quality and the thermal environment were regulated by opening naturally ventilated windows.

Both females and males can be affected by ringworm, but only cocks were affected with a high prevalence. Specifically, 46.7% of all cocks in the flock were affected. The combs of the affected birds showed deposits of thin, white, flour-like matter. In the most severely affected birds, the disease spread concentrically, and the white patches began to flake off, giving the appearance of a wrinkled crust. There were also nodules of soft texture, loosely attached to the feathers, varying in color from white to light brown, mainly on the face and neck, but no systemic signs of disease were observed (Figure 1).

Figure 1: The combs of the affected birds showed deposits of thin, white, flour-like material. There were also nodules of soft texture, loosely attached to the feathers, varying in colour from white to light brown, mainly on the face and neck.

Scrapings from the affected areas of some birds were cultured on Sabouraud dextrose agar supplemented with chloramphenicol to eliminate bacteria. All cultures were incubated at 28 ºC for up to 4 weeks, which gave pure growth of the pathogen. Colonies were slow growing, button or disc-shaped, white to cream in color, with a powdery to suede-like surface, becoming rough and cerebriform after a few days, with a yellow pigmented reverse (Figure 2).

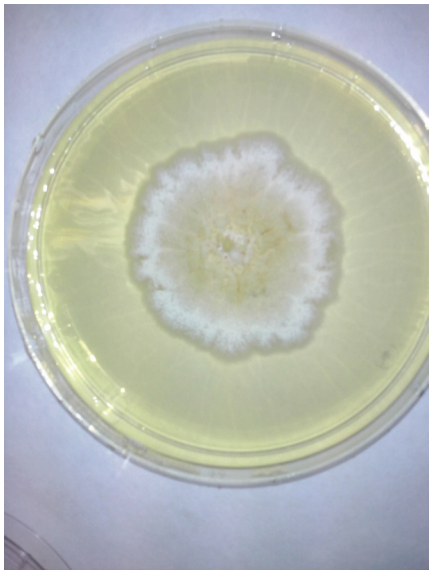

Figure 2: Culture of *Trichosporon jirovecii* on Sabouraud agar medium showing the typical glabrous, heaped and folded white coloured colony.

The isolates were also examined microscopically following staining with lactophenol cotton blue, where septate hyaline hyphae with abundant arthroconidia and blastoconidia were observed (Figure 3).

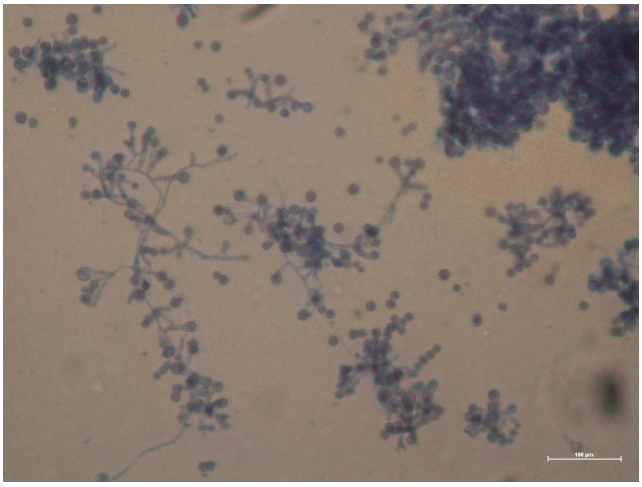

Figure 3: Microscopic image at 40× magnification. Segmented mycelia formed a large number of circular arthrospores.

Phenotypic identification methods are useful for screening *Trichosporon* species, but only molecular methods, such as internal transcribed spacer (ITS) region sequencing, allow the complete identification of *Trichosporon* isolates at the species level (Colombo *et al.*, 2011).

Direct sequencing of polymerase chain reaction products obtained by amplification with the universal fungal primers (ITS1 5'-TCCGTAGGTGAACCTGCGG-3/ ITS4 5'-GCATATCAATAAGCGGAGGA-3') (White et al., 1990) was carried out. A BLAST nucleotide search against the GenBank database revealed that the 520-bp (100 % Identity) nucleotide sequence of the amplified product matched the ITS regions of fungal ribosomal DNA (rDNA) of *Trichosporon jirovecii* Sequence ID: AM900367.1 (Figure 4).

The peak for favus in the outbreak area was March, since ringworm is more common in cold months with low humidity and low rainfall. Another important factor could be the immune status of the host. The control of fungal infection depends initially on the host's immune response. In the case described here, although the housing conditions were similar between hens and cocks, the outbreak of ringworm affected only the males, most likely as a consequence of higher stress levels due to their competitive and dominant behaviour. This stress was even more likely when they were handled weekly for semen collection training by the abdominal massage method (Burrows & Quinn, 1935). Under natural conditions, males (compared to female chickens) have more ability to transmit disease, and especially in fungal disease, to others (Queiroz & Cromberg, 2006). Infection is also favoured by housing in warm, damp, dirty barns with thick layers of manure.

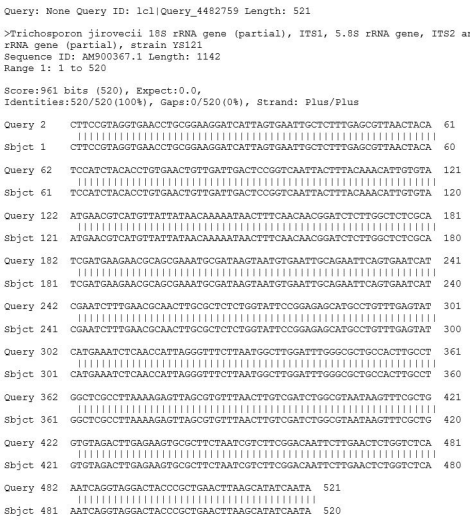

Figure 4: A BLAST nucleotide search against the GenBank database revealed that the 520-bp (100 % Identity) nucleotide sequence of the amplified product matched the ITS regions of fungal ribosomal DNA (rDNA) of *Trichosporon jirovecii* Sequence ID: AM900367.1

Humans are usually infected with favus by animals, usually through direct contact or contaminated fomites. The clinical presentation of *Trichosporon* infection in humans is often benign superficial lesions of hair, called white piedra, characterized by the presence of irregular nodules on the affected hair. These nodules are loosely attached to the hair shaft, have a soft texture, and are white or light brown. Although generally associated with this superficial infection, *Trichosporon* has gained importance as one of the most opportunistic systemic fungal infections, capable of causing life-threatening invasive diseases, especially in immuno-suppressed patients and rarely in immuno-competent patients. Disseminated trichosporonosis can affect a wide array of organs and the presentation can vary from brain abscess, meningitis, endophthalmitis, pneumonia, soft tissue lesions, lymphadenopathy, endocarditis, arthritis, oesophagitis, liver, and splenic abscesses or even uterine infections (Colombo et al., 2011). Treating patients with trichosporonosis remains a challenge because of limited data on the *in vitro* and *in vivo* activities of antifungal drugs against clinically relevant species of the genus. Importantly, all *Cutaneotrichosporon* species are resistant to echinocandins and other classes of antifungal agents (Arendrup et al., 2014).

Clotrimazole is generally considered to be a fungistatic that belongs to the group of imidazole antifungals. Imidazoles inhibit the production of a substance, called ergosterol, which is a component of the fungal cell wall. Damage to the membrane cannot be repaired and this affects the cell's ability to reproduce. Superficial treatment with a clotrimazole-based topical cream has been found to be successful for the most severe favus cases, but milder cases usually clear up without treatment. Some research has shown that infections tend to clear up spontaneously after 10 weeks. (Böhm &

Sasu, 1973). This medication was applied directly to the affected areas of the birds in this study, but removal of scales and crust before applying the cream is recommended.

The spread of infection occurs in birds by direct contact or via contaminated fomites (Droual et al., 1991; Londero et al., 1969). Control measures, such as decontamination of premises and removal and treatment of infected individuals (avoidance of handling uninfected animals after handling infected ones), have been established to reduce the number of organisms present on cocks and to reduce the pathogen load already present in the cockerel house.

Birds with favus should be isolated to prevent transmission of the agent. Biosecurity measures should be taken to prevent the introduction of infected birds into the flock. Transport crates and other equipment should be thoroughly decontaminated and disinfected to prevent lateral transmission of the agent. Care should be taken when handling poultry with favus to prevent zoonotic transmission.

DISCUSSION

This microorganism has been classically associated with superficial infection. However, during recent decades, it has also been linked to infections spread in immunocompromised patients, behaving as an opportunist agent. It is important to know that *Trichosporon jirovecii* can be transmitted to humans by direct contact with an infected animal or via contaminated fomites. For these reasons, the zoonotic risk should be taken into account when handling chickens or their equipment (cages, feed, etc.).

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Authors' contributions

Both authors contributed equally to the writing of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical statement

The flock owner gave his consent for the publication of this case report.

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EPIZOOTIJA PIEDRA BLANCA U JATU EXTREMENA PLAVIH KOKOŠAKA

García ALFREDO, Barraso CARMEN

Kratak sadržaj

Rad opisuje slučaj infekcije izazvane gljivicom *Cutaneotrichosporon jirovecii*, u malom jatu *Extremena* plavih kokošaka, i ističe da bi se bolest mogla pojaviti u obliku epizootije, s obzirom na to da je inficirano 46,70% petlova u jatu. Inficirane ptice su imale bele kraste na kresti, a kod većine inficiranih životinja primećeni su beli ili svetlosmeđi čvorići koji su se labavo vezivali za vrh perja, ali nisu primećeni znaci sistemske bolesti. Dijagnoza *Trichosporon jirovecii* postavljena je morfološkom identifikacijom, a potvrđena je sekvenciranjem amplifikovanog produkta PCR reakcije.

Ključne reči: epizootija, *ringworm*, petao, površinska lezija, *Trichosporon jirovecii*, zoonoza