

Outbreak due to *Fusarium solani* on a Brazilian ostrich farm

Surto por *Fusarium solani* em uma fazenda brasileira de avestruzes

Brote de *Fusarium solani* en una granja brasileña de avestruces

Received: 01/08/2022 | Reviewed: 01/17/2022 | Accept: 01/18/2022 | Published: 01/19/2022

Henri Donnarumma Levy Bentubo

ORCID: <https://orcid.org/0000-0003-0091-2504>

Universidade Paulista, Brazil

Universidade do Vale do Paraíba, Brazil

E-mail: hbentubo@yahoo.com.br

Vivian Ferreira da Silva

ORCID: <https://orcid.org/0000-0001-6816-769X>

Universidade de São Paulo, Brazil

E-mail: vivis_sl@yahoo.com.br

Maria Bárbara Baptista Capellos Daruiz Mangiaterra

ORCID: <https://orcid.org/0000-0001-7263-9338>

Ostrich's Applied Biotechnology, Brazil

E-mail: mabbcdman@usp.br

Sarah Santos Gonçalves

ORCID: <https://orcid.org/0000-0003-4170-4445>

Universidade Federal do Espírito Santo, Brazil

E-mail: sarah.tavares@ufes.br

Abstract

The aim of this study was to present and discuss the laboratory finding of *Fusarium solani* as opportunistic agent of invasive mycosis in chicks and eggs on an ostrich farm located in Cosmópolis (São Paulo, Brazil). Dyspnea and tachypnea were the main signs and symptoms presented by the chicks. Multiple whitish foci were found scattered over the chicks' air sac membranes, lungs and livers and over the external membranes of the eggs. Fungal mycelia were observed in the air compartments of some of the eggs. An isolate purified from a lung culture was subsequently identified as *Fusarium solani* based on its morphological and molecular profiles. It was likely that the high humidity and temperature, as well as the matte paint that had been applied to the hatchery and birth room walls, provided conditions favoring fungal growth. Strict environmental sanitary measures are necessary to maintain microbial control. Uncommon fungi such as *Fusarium solani* must be considered as opportunistic pathogens in commercial ostrich culturing.

Keywords: *Fusarium solani*; Mycotic infection; Ostrich; *Strutio camelus*; Hatchery.

Resumo

O objetivo deste estudo foi apresentar e discutir o diagnóstico laboratorial de *Fusarium solani* como agente oportunista de micose invasiva em filhotes e ovos em uma fazenda de avestruzes localizada em Cosmópolis (São Paulo, Brasil). Dispneia e taquipneia foram os principais sinais e sintomas apresentados pelos filhotes. Vários focos esbranquiçados foram encontrados espalhados sobre as membranas do saco aéreo dos pulmões e fígados dos filhotes e sobre as membranas externas dos ovos. Fungos filamentosos foram observados na câmara de ar de alguns dos ovos. Um isolado purificado de uma cultura de pulmão foi posteriormente identificado como *Fusarium solani* com base em seus perfis morfológicos e moleculares. Provavelmente, a alta umidade e temperatura, bem como, a tinta fosca aplicada nas paredes do incubatório e da sala de eclosão proporcionaram condições favoráveis ao crescimento desses fungos. A adoção de medidas sanitárias ambientais mais rigorosas deverá manter o controle microbiano. Fungos incomuns, como *Fusarium solani*, devem ser considerados patógenos oportunistas na cultura comercial de avestruzes.

Palavras-chave: *Fusarium solani*; Infecção micótica; Avestruz; *Strutio camelus*; Incubação.

Resumen

El objetivo de este estudio fue presentar y discutir el diagnóstico de laboratorio de *Fusarium solani* como agente oportunista de micosis invasora en pollos y huevos de una granja de avestruces ubicada en Cosmópolis (São Paulo, Brasil). La disnea y la taquipnea fueron los principales signos y síntomas que presentaron los pollos. Se encontraron varios focos blanquecinos esparcidos sobre las membranas del saco de aire de los pulmones e hígados de las crías y sobre las membranas externas de los huevos. Se observaron hongos filamentosos en la cámara de aire de algunos de los huevos. Un aislado purificado de un cultivo de pulmón se identificó posteriormente como *Fusarium solani* basándose en sus perfiles morfológicos y moleculares. Probablemente, la alta humedad y temperatura, así como la pintura mate aplicada a las paredes de la incubadora y la sala de incubación, proporcionaron condiciones favorables

para el crecimiento de estos hongos. La adopción de medidas sanitarias ambientales más estrictas debe mantener el control microbiano. Los hongos inusuales, como *Fusarium solani*, deben considerarse patógenos oportunistas en el cultivo comercial de avestruces.

Palabras clave: *Fusarium solani*; Infección micótica; Avestruz; *Strutio camelus*; Incubación.

1. Introduction

The ostrich (*Struthio camelus*) is the largest living bird in existence. This rustic producer of meat, eggs, leather and feathers has achieved a privileged position in the Brazilian agribusiness market in a single decade (Almeida et al, 2005). The greatest difficulties in ostrich culture have been observed during the reproductive phase, which is the period from egg deposition to 90 days-old, during the unhatched and young birds are particularly susceptible to infectious diseases (Cooper et al, 2009).

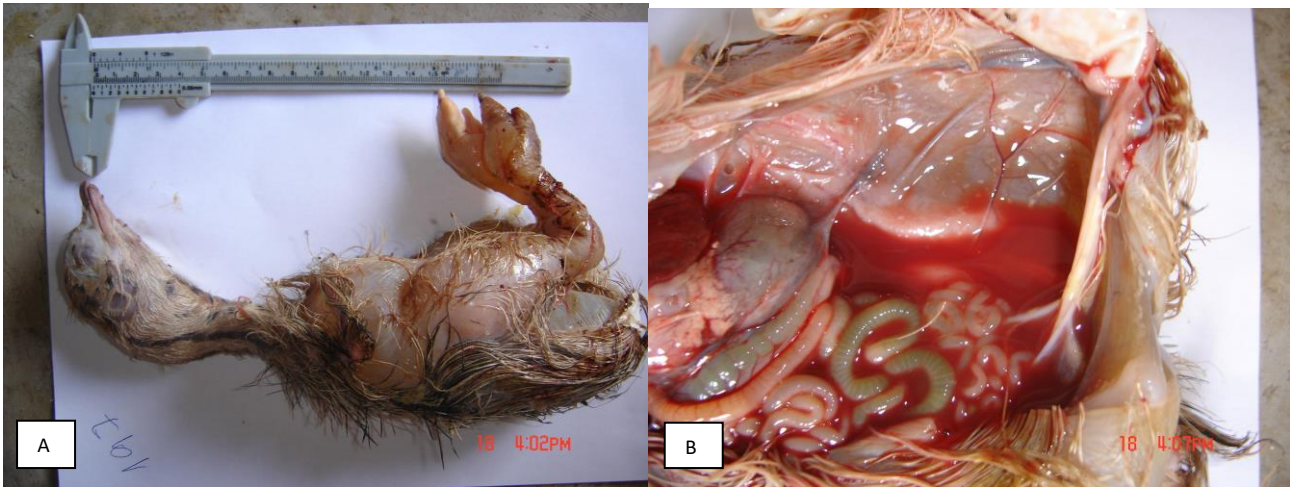
The management practices and the health of the ostriches are the most important factors in controlling the spread of disease and achieving success in farming (Evans et al, 2004; Andreatti-Filho, 2000). Fungi may be responsible for serious cases of intoxication and infection that are frequently fatal for birds, especially the more predisposed individuals, such as chicks. The *Aspergillus* genre is the most common fungal pathogen identified in invasive avian mycosis. However, the new husbandry production methods have increasing the opportunistic infections caused by uncommon agents (Gulbahar et al, 2000; Gonçalves et al, 2012).

The aim of this paper was to discuss the identification of *Fusarium solani* as opportunistic agent of invasive mycosis in eight ostrich chicks and eggs at a Brazilian commercial ostrich farm located in Cosmópolis (São Paulo, Brazil) which were showing declining production due to recurrent fungal infections with fatal outcome of eggs and neonates. The cases were two newborn chicks and six eggs containing fetuses that were in the final stages of incubation, all from a commercial breeding facility located in Cosmópolis (Sao Paulo, Brazil). The present article characterizes a qualitative case study and its development is supported by the following scientific methodology proposed on Estrela (2018), Pereira et al (2018), Yin (2015) and Ludke and André (2013).

2. Case Reported

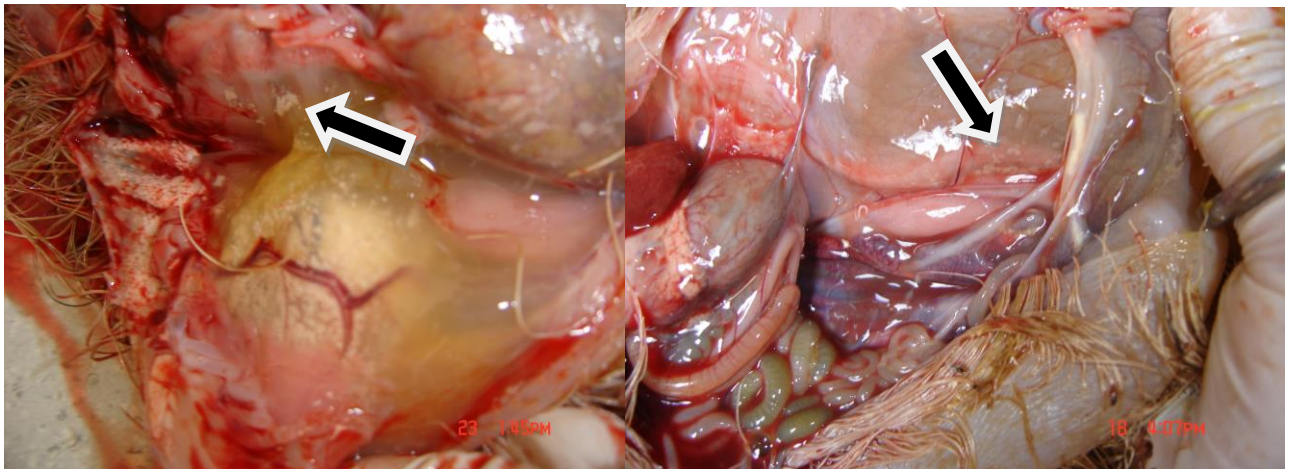
The two chicks hatched spontaneously but presented clinical signs of acute respiratory distress within hours of hatching. Intense dyspnea and tachypnea, followed by sudden death (Figure 1A), were the main signs. To confirm fetal death in the eggs that exhibited delayed emergence, they were removed from the hatchery and opened. During the necropsies of the unhatched chicks, blood was observed in their coelomic cavities (Figure 1B). In addition, numerous whitish spots were distributed throughout their coelomic cavities, on the air sac membranes, livers and lungs (Figure 2). Whitish spots, similar to those observed in the chicks, were observed on the outer membrane of the eggs. A grayish powdery material occupied a large portion of the air chamber in one of the eggs. Tissue samples (air sac membrane, liver and lung) were collected from a chick and one egg. All of the samples were sent to the reference veterinary laboratory in the Campinas region, in São Paulo (Brazil). The samples were seeded onto nutrient agar plates and incubated at 37°C for 24 hours for bacterial evaluation, and they were seeded onto Sabouraud dextrose agar plates and incubated at room temperature for five days for fungal evaluation. However, new fungal tests were requested by the veterinarians, so tissue samples were sent to where they were cultured on brain heart infusion (BHI) agar plates and incubated at 30°C.

Figure 1. Outbreak of *F. solani* infection in an ostrich farm in the region of Cosmópolis, SP (Brazil). In "A", is observed an ostrich chick that died at birth. In "B", there is hemorrhage in the coelomatic cavity on another bird.



Source: Authors' personal archive.

Figure 2. Outbreak of *F. solani* infection in an ostrich farm in the region of Cosmópolis, SP (Brazil). Numerous whitish spots were distributed throughout their coelomic cavities, on the air sac membranes, livers and lungs of the animals.



Source: Authors' personal archive.

The clinical isolate was identified as *Fusarium solani* based on its morphological and molecular profiles. Traditional methods were used to conduct the morphological study at Laboratory of Molecular and Cell Biology (UNIP), in São Paulo, SP (Brazil). For the molecular characterization, developed at Filamentous Fungi Division, Especial Laboratory of Mycology, Federal University of São Paulo (UNIFESP), São Paulo, SP (Brazil), the isolate was grown on yeast extract-sucrose agar (YES; 10 g of yeast extract, 75 g of sucrose and 10 g of agar per 500 ml of H₂O). DNA was extracted using the PrepMan™ Ultra Sample Preparation Reagent (Applied Biosystems™, Life Technologies™, Foster City, CA, USA) protocol. Its concentration and purity (with respect to protein and salts) were determined from the optical density at 260 nm and the O.D. 260/280 nm and O.D. 260/230 nm ratios, respectively. Primers ITS1 and ITS4 were used to amplify the ITS region of the rDNA. The reactions were performed according to the PCR SuperMix High Fidelity (Invitrogen™ Life Technologies™, Foster City, CA, USA) protocol. The amplification program included an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C and extension for 1 min at 72°C. A final extension step at 72°C for 7 min

was included at the end of the amplification. After amplification, the fragments were sequenced following the protocol provided with the BigDye reagent kit (Applied Biosystems™ Life Technologies™, Foster City, CA, USA). The ITS regions were sequenced using the PCR primers. Consensus sequences were obtained using AutoAssembler (Applied Biosystems™ Life Technologies™, Foster City, CA, USA) and SeqMan™ software (Lasergene™, Madison, WI, USA). These sequences were subjected to a BLAST analysis (BLASTn) for species identification using two different databases: the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) and the Centraalbureau voor Schimmelcultures (CBS) (<http://www.cbs.knaw.nl/Collection/BioloMICSSequences.aspx?file=all>) databases.

3. Discussion

Fungi are ubiquitous microorganisms, and their proliferation is highly influenced by environment factors, as high temperature and humidity. The farm where the outbreak occurred had received an international certificate of good management practices (ISO-9001). However, the walls of the incubator and the hatchery facilities were coated with matte white paint, which most likely did not permit adequate hygiene and sanitary management, eventually predisposing these units to the colonization and proliferation of undesirable pathogens. Microbial agents can be introduced into aviculture incubators and hatcheries through contaminated eggs, insects and/or inappropriately dressed employees, posing a considerable risk of infection for the chicks (Katz et al, 1996; Jeffrey et al, 1994; Khosravi et al, 2008).

High concentrations of microbial agents, even those of low pathogenicity, constitute an important risk factor for infection in animals and humans. Colonization of the cloaca, for example, can facilitate the contamination of the shells of laid eggs and is associated with infectious processes in the yolk sac and embryonic death. Immunosuppressive factors, such as young age, stress and antibiotic administration, aggravate opportunistic infections. *Candida albicans*, *Rhodotorula* spp. and *Cryptococcus* spp. have been identified in the normal microbiota of the oropharynges and cloacae of ostriches (Cooper et al, 2009; Lábague et al, 2003; Evans et al, 2004). While attempting to isolate filamentous fungi from the oropharynges and feces of ostriches obtained negative results; however, possible cases of pulmonary infection by these agents should be considered because their lower respiratory tracts are particularly affected by the inhalation of propagules present in the environment. No specific studies that provide information regarding the diversity of the fungal microbiota of the ostrich body surface are available (Almeida et al, 2005).

Opportunistic fungal infections can affect various segments of animal husbandry. *Aspergillus fumigatus* is the mycelial fungus that is most frequently associated with embryonic death and with respiratory symptoms in neonates in aviculture (Lima et al, 2001; Khosravi et al, 2008). The acute respiratory form, which mainly affects the lungs and air sacs, is the clinical form most frequently observed in birds with aspergillosis. In the case of chickens less than two-weeks old, which are more susceptible than older chicks to this acute form of pneumonia, the rates of morbidity and mortality are generally quite high (Melville et al, 2004; Oliveira et al, 2006; Khosravi et al, 2008). The presentation of a clinical pulmonary infection caused by *A. fumigatus* is very similar to that produced by other filamentous fungi, such as *Paecilomyces* spp., *Acremonium* spp., *Trichoderma* spp. and *Scopulariopsis* spp., which are common in the environment but are rarely identified as avian pathogens (Oliveira et al, 2006; Gonçalves et al, 2012). Reports of proventriculitis and ulcerative ventriculitis in ostriches have been linked to the existence of granulomatous pneumonia caused by *Mucor* spp. (Reissig et al, 2004; Orós et al, 2004). In this study, the clinical presentation of the affected ostriches was similar to that described for aspergillosis in birds in other countries (Richard, 1997); however, this investigation indicated that *F. solani* was the etiologic agent.

In one (16.6%) of the six unhatched eggs, a grayish powdery material occupied a large portion of the air chamber. This contamination was most likely attributable to the presence of the fungus in high concentrations in the facilities. The

material, denominated a fungal mat and consisting of an accumulation of fungal structures, was located between the outer and inner layers of the air chamber. These findings are consistent with those observed by others in cases of infection by non-*Fusarium* mycelial fungi (Gonçalves et al, 2012).

Fusarium solani has been described as an infectious agent in reptiles, fish and mammals. Severe multifocal granulomatous pneumonia caused by *F. solani* was observed in a sea turtle (*Lepidochelys kempi*) specimen (Smith et al, 1989). Fatal infections by *F. solani* were also diagnosed in shark pups (*Sphyrna tibur*) that were born in the National Aquarium in Baltimore (Uhart et al, 2006). In a case of meningoencephalitis in a German Shepherd dog, *F. solani* was the only agent identified (Yokota et al, 2004). Immaturity, advanced age, malnutrition, chronic underlying disease, stress or any other disorder that directly or indirectly affects the balance of an animal's immune system can facilitate infections by microorganisms that are uncommon disease agents, such as *Fusarium* spp. (Khosravi et al, 2008).

Companies involved in animal production, being concerned with productivity and potential economic losses, have adopted measures for the increasingly strict control of pathogens within aviculture incubators and hatcheries. It is important to monitor the environmental fungal load in facilities where the risk of infection is high. The signs and symptoms observed in this study were suggestive of an invasive mycotic process. The isolation of *F. solani* from the ostrich chicks and infected eggs is a strong indication that this organism was the etiologic agent. *Fusarium solani* fungus is commonly found in the environment and sometimes is identified in opportunistic invasive infections in humans, but is rarely observed in animal infections, although that trend may change in the near future (Gulbahar et al, 2000).

Because ostrich farming was only recently introduced into Brazil, little is known regarding the biological and clinical aspects of this species in this country. Knowledge of the normal microbiota of these birds and the environmental fungal load, as well as the molecular genotyping of clinical and environmental isolates will facilitate future investigations of the less frequently reported opportunistic fungi, such as *Fusarium solani*, to guide the adoption of adequate sanitary measures to prevent new outbreaks.

4. Final Considerations

Because ostrich farming was only recently introduced into Brazil, little is known regarding the biological and clinical aspects of this species in this country. Knowledge of the normal microbiota of these birds and the environmental fungal load, as well as the molecular genotyping of clinical and environmental isolates will facilitate future investigations of the less frequently reported opportunistic fungi, such as *Fusarium solani*, to guide the adoption of adequate sanitary measures to prevent new outbreaks.

References

- Almeida, R. M., Bianchi, M. D., Gonçalves-Neto, M. C., Souza, R. R., Campos, W. R. (2005). Microbiota da orofaringe e fezes de avestruzes (*Struthio camelus*) clinicamente sadios: estudos preliminares [Microbiota of the oropharynx and feces of clinically normal ostriches (*Struthio camelus*): Preliminary studies]. *Boletim Medicina Veterinária*. 1:49–56.
- Andreatti-Filho R. L. (2000). *Doenças das Aves* [Poultry Disease]. In: A. Berchieri-Júnior and M. Macari, (Eds.). *Enfermidades micóticas*, pp 369–378 (Campinas, FACTA).
- Cooper, R. G., Mahrose, K. M., Horbańczuk, J. O., Villegas-Vizcaíno, R., Kennou-Sebei, S. (2009). The wild ostrich (*Struthio camelus*): A review. *Tropical Animal Health and Production*. 41:1669–1678.
- Estrela, C. (2018). *Metodologia científica: ciência, ensino, pesquisa*. Artes Médicas.
- Evans, J., Levesque, D., Lahunta, A., Jensen, H. E. (2004). Intracranial fusariosis: A novel cause of fungal meningoencephalitis in a dog. *Veterinary Pathology*. 41:510–514.
- Gonçalves, S. S., Cano, J. F., Stchigel, A. M., Melo, A. S., Godoy-Martinez, P. C., Correa, B., Guarro J. (2012). Molecular phylogeny and phenotypic variability of clinical and environmental strains of *Aspergillus flavus*. *Fungal Biology*. 116:1146–1155.

- Gulbahar, M. Y., Agaoglu, Z., Biyik, H., Yuksek, N. (2000). Zygomycotic proventriculitis and ventriculitis in ostriches (*Struthio camelus*) with impaction. *Australian Veterinary Journal*. 78:747–749.
- Jeffrey, J. S., Chin, R. P., Shivaprasad, H. L., Meteyer, C. U., Droual, R. (1994). Proventriculitis and ventriculitis associated with zygomycosis in ostrich chicks. *Avian Diseases*. 38:630–634.
- Katz, M. E., Love, S. C., Gill, H. S., Cheetham, B. F. (1996). Development of a method for the identification, using the polymerase chain reaction, of *Aspergillus fumigatus* isolated from ostriches. *Australian Veterinarian Journal*. 74:50–54.
- Khosravi, A. R., Shokri, H., Ziglari, T., Naeini, A. R., Mousavi, Z., Hashemi, H. (2008). Outbreak of severe disseminated aspergillosis in a flock of ostrich (*Struthio camelus*). *Mycoses*. 51:557–559.
- Lábaque, M. C., Navarro, J. L., Martella, M. B. (2003). Microbial contamination of artificially incubated Greater Rhea (*Rhea americana*) eggs. *British Poultry Science*. 44:355–358.
- Lima, J. S. J., Pinto, D. M., Carrasco, L.O., Salgueiro, F. J., Meireles, M. C. (2001). Incidência de fungos na produção de pintos de corte de um dia de idade. *Revista Brasileira de Agrociência*. 7:73–77.
- Ludke, M., & André, M. (1986). Pesquisa em educação: abordagens qualitativas. *Em Aberto*, 5(31).
- Melville, P. A., Cogliati, B., Mangiaterra, M. B., Peres, M. R., Moura, S. C., Matsuda, L. (2004). Determinação da microbiota presente na cloaca e orofaringe de avestruzes (*Struthio camelus*) clinicamente saudáveis. *Ciência Rural*. 34:1871–1876.
- Oliveira, G. R., Ribeiro, J. M., Fraga, M. E., Cavaglieri, L. R., Direito, G. M., Keller, K. M., Dalcero, A. M., Rosa C. A. (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia* 162:355–362.
- Orós, J., Delgado, C., Fernández, L., Jensen, H. E. (2004). Pulmonary hyalohyphomycosis caused by *Fusarium* spp. in a Kemp's Ridley sea turtle (*Lepidochelys kempi*): An immunohistochemical study. *New Zealand Veterinary Journal*. 52:150-152.
- Pereira, A. S., Shitsuka, D. M., Parreira, F. J., & Shitsuka, R. (2018). *Metodologia da pesquisa científica*. UFSM. https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf.
- Reissig, E. C., Terzolo, H., Armando, S., Rogé, A. (2004). Hatching success and embryonic mortality on lesser rhea (*Pterocnemia pennata*) farms in northern Patagonia, Argentina. *British Poultry Science*. 45:471–475.
- Richard, J. L. (1997). *Aspergillosis*. In: B.W. Calnek, (Ed.). *Diseases of Poultry*, pp. 351–360 (Iowa, Iowa State University Press).
- Smith, A. G., Muhvich, A.G., Muhvich, K.H., Wood, C. (1989). Fatal *Fusarium solani* infections in baby sharks. *Journal of Medical and Veterinary Mycology*. 27: 83–91.
- Uhart, M., Aprile, G., Beldomenico, P., Solís, G., Marull, C., Beade, M., Carminati, A., Moreno, D. (2006). Evaluation of the health of free-ranging greater rheas (*Rhea americana*) in Argentina. *Veterinary Records*. 158:297–303.
- Yin, R. K. (2015). *Estudo de caso: planejamento e métodos*. Porto Alegre.
- Yokota, T., Shibahara, T., Wada, Y., Hiraki, R., Ishikawa, Y., Kadota, K. (2004). *Aspergillus fumigatus* infection in an ostrich (*Struthio camelus*). *Journal of Veterinary Medical Science*. 66:201–204.