



## An outbreak of avian pox in greater rheas (*Rhea americana*) in Northeast Brazil<sup>1</sup>

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**ABSTRACT.** - Eloy L.R.C., Linhares M.T., Sousa M.S., Lacerda W.K., Sousa I.V. & Lucena R.B. 2024. **An outbreak of avian pox in greater rheas (*Rhea americana*) in Northeast Brazil.** *Pesquisa Veterinária Brasileira* 44:e07560, 2024. Graduate Program in Animal Science, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Campus II, PB-079 Km 12, Areia, PB 58397-000, Brazil. E-mail: [lireloy@gmail.com](mailto:lireloy@gmail.com)

Greater rheas (*Rhea americana*) are Brazil's largest non-flying bird species. Currently, they are listed as "Near Threatened" on IUCN's Red List of Endangered Species, mainly because of animal trafficking, deforestation and illegal hunting. Greater rheas' diseases are poorly documented in Brazil, although they are a native species, and there are conservational scientific facilities that keep and reproduce them. We report an outbreak of avian pox in 13 juvenile greater rheas (*Rhea americana*) in Northeast Brazil. All 13 rheas infected with the virus developed clinical signs associated with respiratory disease, such as serous nasal discharge; they were gasping and breathing with their beaks open. Severe whitish, chronicle and round-crusts multifocal to coalescent nodules formed on their skin. Out of the 13 affected rheas, nine (69%) died a few weeks after the first clinical signs. Only four (31%) of the rheas survived after unspecified treatment with antibiotics. Two rheas were referred to necroscopic evaluation at the "Laboratório de Patologia Veterinária" of "Universidade Federal da Paraíba", Campus II, Areia. Microscopically, acantholytic lesions, hyperkeratosis, heterophilic infiltrate and the presence of Bollinger bodies, the pathognomonic inclusion body caused by poxvirus, were seen in the skin. Thus, the avian pox diagnosis was confirmed by clinical and histopathological examinations. This study seems to be the first on avian pox in greater rheas in Northeast Brazil. Investigating the causes of death of wild native birds is essential to the preservation of species. Greater rheas are an important part of the ecosystem and suffer from illegal hunting and animal trafficking. Necroscopic examination allows us to detect pathogens and take prophylactic measures to avoid the decreasing number of wild populations.

INDEX TERMS: Avian poxvirus, ratites, greater rheas, *Rhea americana*, conservation, histopathology.

**RESUMO.** - [Surto de boubá aviária em emas (*Rhea americana*) no Nordeste do Brasil.] As emas (*Rhea americana*) são a maior espécie de ave não-voadora do Brasil. Atualmente, estão listadas como "Quase Ameaçada" na Lista Vermelha das Espécies Ameaçadas da União Internacional para a Conservação da Natureza e dos Recursos Naturais, principalmente devido

ao tráfico animal, desmatamento e caça ilegal. As doenças das emas são pouco documentadas no Brasil, apesar de serem uma espécie nativa e existirem centros científicos de conservação que as mantêm e as reproduzem. Relatamos um surto de boubá aviária em 13 emas (*Rhea americana*) juvenis no Nordeste do Brasil. Todas as 13 emas infectadas com o vírus desenvolveram sinais clínicos associados à doença respiratória, como secreção serosa nasal, estavam ofegantes e respirando com os bicos abertos. Na pele, formaram-se severos nódulos brancos, crônicos, crustosos, arredondados, multifocais a coalescentes. Das 13 emas afetadas, nove (69%) morreram em poucas semanas após os primeiros sinais clínicos. Apenas quatro emas (31%) sobreviveram após tratamento inespecífico com antibióticos. Duas emas foram

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encaminhadas para exame necroscópico no Laboratório de Patologia Veterinária da Universidade Federal da Paraíba, Campus II, Areia. Microscopicamente, foram observadas lesões acantolíticas, hiperqueratose, infiltrado heterofílico e a presença de Corpos de Bollinger, corpúsculos de inclusão patognomônicos do poxvírus. Portanto, o diagnóstico de boubá aviária foi confirmado após os exames clínicos e histopatológicos. Este parece ser o primeiro relato de boubá aviária em emas no Nordeste do Brasil. Investigar as causas de morte de aves silvestres é essencial para a preservação das espécies. Emas possuem um papel importante no ecossistema que habitam e sofrem com a caça ilegal e o tráfico animal. A necropsia nos permite detectar patógenos e tomar medidas profiláticas para evitar a diminuição de populações selvagens.

TERMOS DE INDEXAÇÃO: Poxvírus aviário, ratitas, emas, *Rhea americana*, conservação, histopatologia.

## INTRODUCTION

Avian pox is common among poultry and reported in several countries. It also affects companion birds and wild species of birds. It is caused by the double-stranded DNA virus *Avipoxvirus fowlpox* from the genus *Avipoxvirus*, which has already been identified in over 270 species of birds (Delhon 2022). There are two forms of infection by avian poxvirus: the cutaneous and the diphtheric form. Cutaneous lesions consist of proliferative nodules, mainly on the non-feathered parts of the body. Diphtheric lesions are usually proliferative fibronecrosis in mucous membranes of the respiratory and gastrointestinal tract (Swayne et al. 2020). The virus targets keratinocytes, causing hyperplasia of the epidermis and hyperkeratosis of the dermis, ballooning degeneration, and intracytoplasmic eosinophilic inclusion bodies called Bollinger bodies; these lesions are pathognomonic for avian poxvirus infections (Shivaprasad & Barnes 2016, Delhon 2022). Diagnosis may be also confirmed if the virus DNA is extracted, polymerase chain reaction (PCR) amplified and sequenced (Lee & Lee 1997, Kunert-Filho et al. 2016). Factors that increase the distribution and prevalence of the infection include weather conditions, the presence of vectors, the density and susceptibility of hosts and the presence of poxvirus. Transmission might happen through lesioned skin, especially by mosquitoes or ectoparasites, such as mites. However, it can also be transmitted directly by contact with infected birds or contaminated objects and surfaces (Van Riper III & Forrester 2007). It is an infection of economic and conservational significance since the mortality rate might be high if there are complications, poor environmental conditions or other diseases in association (Swayne et al. 2020, Silva et al. 2023).

Greater rheas (*Rhea americana*) are South America's largest non-flying bird species. They are considered "Near Threatened" in IUCN's Red List of Threatened Species (BirdLife International 2022), mainly due to deforestation, illegal hunting and predation. Poxvirus infection was reported in rheas in Spain in 1938 (Volgensag 1938), but there are no reports of this infection in Brazil. The health of 22 free-ranging rheas was also evaluated, and antibodies to several infectious agents were identified, but no antibodies for avian pox were found (Uhart et al. 2006). Rheas are commercially farmed for meat, hide, feathers and eggs and maintained in captivity for conservational and academic research (Kummrow 2015, Coogan 2021). The aim of this study was to report an outbreak of avian pox in juvenile greater rheas in Brazil, emphasizing its clinical and histopathological findings.

## MATERIALS AND METHODS

**Ethical approval.** The use of the rheas' carcasses for this scientific study was submitted to the "Sistema de Autorização e Informação em Biodiversidade" (SISBIO) of the "Instituto Chico Mendes de Conservação da Biodiversidade" (ICMBIO), registration number 94079-1.

**Data collection.** The rheas were farmed on a ranch in Northeast Brazil. The case medical history was collected and consisted of their origin, how many rheas had been introduced into the ranch, when they arrived, where they were housed, if there were any contactants of other species, the development of the disease, how many were affected, how many died, if they were treated and what kind of medicine was used. The cases were divided into Case no.1 and Case no.2. Case no.1 depicts the necropsy of a rhea that died suddenly and had pox-like skin lesions and respiratory clinical signs. Case no.2 regards the necropsy of a rhea that died 30 days after Case no.1's rhea and still presented pox-like skin lesions even after the others that survived in the flock had already improved from these lesions.

**Necropsy and histopathology.** After death, they were necropsied as is recommended for avian species (Jagne & Buckles 2021), and samples of tissues were processed routinely (Feldman & Wolfe 2014).

## RESULTS

### Case history

A flock of 13 juvenile greater rheas was introduced into a ranch in Northeast Brazil. The ranch farmed cattle, horses and backyard poultry, which consisted of chicken, turkeys and guinea fowl. The rheas were housed with the other avian species; in less than a month, eight (61.5%) of the rheas died. All of them (100%) presented nodular lesions over their bodies (Fig.1). The farm's keeper separated the surviving rheas and placed them in a small shed with no direct sunlight incidence and no ventilation (Fig.2). After unspecific treatment with antibiotics, another individual (Case no.1) of the remaining five rheas died and was referred to the "Laboratório de Patologia Veterinária" of the "Universidade Federal da Paraíba" (LPV-UFPB), Campus II, Areia. Thirty days after the death of this



Fig.1-2. Clinical examination of juvenile greater rheas (*Rhea americana*) naturally infected by avian pox in Northeast Brazil. (1) Round multifocal to coalescent proliferative nodules on the skin of the head and neck of the rhea. Note how the external auricular canal is partially obstructed due to crusted lesions. There are also lesions on its eyelids. (2) Five juvenile rheas gather on the corner of the shed where they were housed after the death of the other eight individuals. There was no sunlight incidence or ventilation.

rhea, three of the remaining ones improved in health and no longer had skin lesions, but another one (Case no.2) persisted with lesions, became apathetic, anorectic and died. This rhea was also referred for *post mortem* examination.

### Macroscopy and histopathological findings

Case no.1 rhea (Fig.3) had skin lesions on its face, neck, torso, legs and wings (Fig.4-6). Some nodules developed around the external ear opening, infiltrating the ear canal, on the base of



Fig.3-8. Case no.1. Macroscopic lesions of a juvenile greater rhea (*Rhea americana*) naturally infected by avian pox in Northeast Brazil. (3) Round multifocal to coalescent proliferative nodules on the skin of the head, neck and torso of the rhea. (4) Nodular crusted lesions on the rhea's head. Note the lesions on the external auricular canal and eyelids. (5) Nodular crusted lesions on the skin of the rhea's left leg. There are lesions both on feathered and unfeathered areas. (6) Poxvirus lesions on the rhea's right wing. There are proliferative nodular crusted formations around feather follicles. (7) Enlargement of the rhea's liver and severe atrophy of the cardiac fat tissue. (8) Congestion of enteric blood vessels and frail yellowish material distributed among viscera compatible with fibrin.

the beak, in the eyelids and on the skin around the outer sheath of wing feathers. The lesions were proliferative, multifocal to coalescent, round, white to yellowish, crusted nodules. Poxvirus lesions were spread throughout the rhea's body except its feet. The rhea had no lesions on its mucous membranes, which suggested the diphtheric form of avian pox. Other macroscopic findings included discrete ascites, severe atrophy of cardiac and ventriculus fat tissue (Fig.7), congestion of enteric blood vessels, an enlarged liver, fibrinous material among viscera, the presence of gas in the intestines (Fig.8), and the ventriculus was filled with foreign objects besides feed and small rocks. Microscopically, the lung had hypertrophy of smooth muscle, heterophilic infiltration and epithelial hyperplasia. There was blood in some parabronchus' lumen. There were no significant histological lesions on the other organs.

Case no.2 necropsied rhea (Fig.9) had similar skin lesions to the ones in Case no.1, but its nodular crusts were thicker and wider, multifocal to coalescent, forming large plaques (Fig.10-11). Also, this rhea was noticeably smaller than the other individuals of the same age. During necropsy, it was observed that the rhea's coelomic cavity was filled with fibrin and the intestines walls were thin, frail and reddish. It was not possible to identify other organs due to the advanced necrotic state in which they were presented. Upon macroscopic examination, a piece of metal was found piercing through the rhea's ventriculus wall, causing traumatic ventriculitis and the intestinal content, mostly cereal grains, from the ventriculus onward, was mostly undigested. In addition to these lesions on the intestinal walls, there was severe acute fibrinous celomitis (Fig.12). Upon microscopic evaluation, the ventriculus had



Fig.9-12. Case no.2. Macroscopic lesions of a second juvenile greater rhea (*Rhea americana*) naturally infected by avian pox in Northeast Brazil. This specimen did not recover from the lesions after a 30-day treatment period and died. (9) Round multifocal to coalescent proliferative nodules on the skin of the head and neck of the rhea. (10) Nodular crusted lesions on the rhea's head. Note the coalescent lesions on the external auricular canal, neck, and eyelids. (11) Nodular crusted multifocal to coalescent lesions on the skin of the rhea's ventral torso. (12) Foreign body found in the rhea's ventriculus. The metallic object was piercing through the ventriculus' wall, and fibrous tissue surrounded the tip of the object.

degenerate and necrotic mucosal epithelial cells. There was also diffuse serositis and necrosis in the other organs.

Avian fowlpox was confirmed in both cases by macroscopic and histopathological analysis based on the observation of typical pox lesions on the skin, such as epithelial hyperplasia and the identification of Bollinger bodies, the pathognomonic intracytoplasmic eosinophilic inclusion bodies found in pox lesions (Fig.13-18).

## DISCUSSION

This study seems to be the first on avian pox in greater rheas in Northeast Brazil. The results shown in this report highlight how deadly poxvirus infections can be to juvenile greater rheas. Both cases' histopathological findings, clinical presentation, and macroscopic lesions were consistent with the cutaneous form of avian pox. The virus aggregates inside infected epidermal cells and forms eosinophilic intracytoplasmic inclusion bodies,

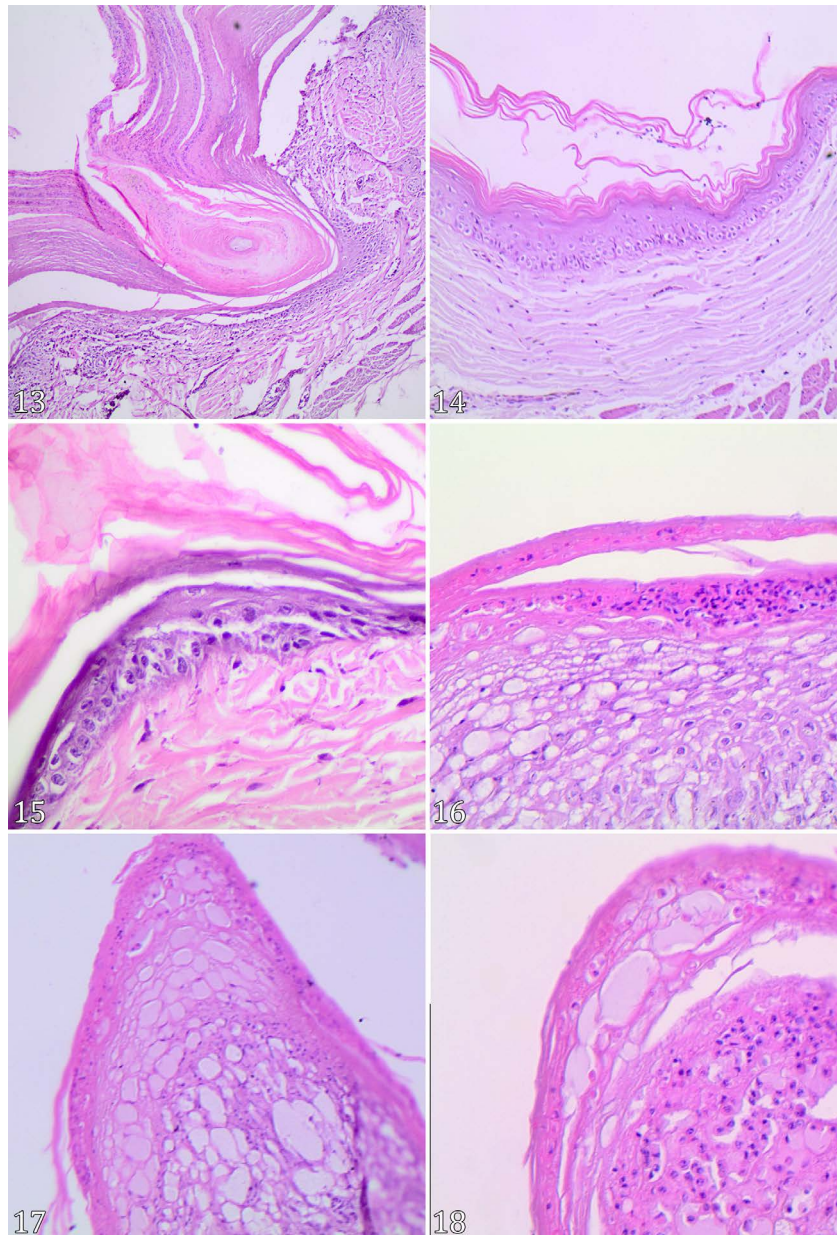


Fig.13-18. Photomicrography of histopathological lesions of avian pox in juvenile greater rheas (*Rhea americana*) in Northeast Brazil. (13-15) Case no.1. (16-18) Case no.2. (13) Nodular lesion surrounding a feather follicle. Severe hyperkeratosis of the epidermal keratinocytes. HE, obj.10x. (14) Nodular acantholytic lesion with heterophilic infiltrate and hyperplasia of the epidermis. HE, obj.20x. (15) Inclusion bodies (Bollinger bodies) are present inside the cytoplasm of keratinocytes of the epidermis. HE, obj.40x. (16) Acantholytic hypertrophy and hyperplasia of epidermal keratinocytes with ballooning degeneration. Note the inclusion bodies in keratinocytes and the heterophilic infiltrate in the deeper layers of the epidermis. HE, obj.40x. (17) Severe ballooning degeneration of the epidermis. Note the inclusion bodies (Bollinger bodies) in keratinocytes. HE, obj.20x. (18) Acantholytic lesion and ballooning degeneration along with an intense heterophilic infiltrate and larger inclusion bodies. HE, obj.40x.

making histopathological diagnosis accurate (Delhon 2022). The disease has already been diagnosed in over 200 species of wild, domestic and companion birds, and its cutaneous form is usually mild and transient (Shivaprasad & Barnes 2016). Avian populations that do not possess a co-evolutionary history with the vectors and the virus are more susceptible and are greatly impacted by the disease. Different species of birds can be infected by pathogenic avian pox strains (Swayne et al. 2020).

The epizootiology is influenced by host density, host susceptibility and the presence of vectors occurring within a certain space and time. In wild birds, Bollinger bodies are not always present as indispensable structures in the development and maturation of avian pox, which means that their histopathological presentation might vary depending on the infected species (Van Riper III & Forrester 2007).

Despite the characteristic skin lesions, there are multiple illnesses that present themselves with similar external wounds and could be listed as differential diagnoses. Diseases that cause proliferative, crusted lesions on the head and body, such as papillomavirus and knemidokoptic mange, can be considered differentials for the cutaneous presentation of avian pox. In contrast, trichomoniasis and capillariasis produce lesions that resemble the diphtheric form of avian poxvirus infection (Van Riper III & Forrester 2007, Weli & Tryland 2011).

The juvenile rheas of the study were exposed to adult turkeys, chickens, and guineafowl, species with documented avian pox cases (Van Riper III & Forrester 2007). Even after vaccination, turkeys present cutaneous and diphtheric forms of avian pox (Ferreira et al. 2018). Although the primary prophylactic measure in commercial poultry is vaccination, for viruses isolated from wild birds, it is difficult to develop specific vaccines since a wide variety of bird species were infected, but not all these poxviruses have been characterized. Thus, research to develop efficient and effective vaccines for wild birds is necessary (Weli & Tryland 2011). There are no vaccines approved by the Brazilian government for rheas, and this increases their susceptibility to infectious pathogens. Furthermore, there was no information provided by the owner regarding the case's history concerning the vaccination of the other species of birds on the ranch. Aside from vaccination, other prophylactic measures when rearing rheas keep them apart from other avian species (Huchzermeyer 2005). There was also no clarification regarding the origin of the juvenile rheas, implying that they were trafficked.

## CONCLUSIONS

We report an outbreak of avian pox in a flock of juvenile greater rheas, highlighting histopathological and clinical findings. Upon microscopic evaluation, the crusted nodules on the rheas' skin revealed epidermal hyperplasia, ballooning degeneration and inclusion bodies in the cytoplasm of keratinocytes (Bollinger bodies).

Poxvirus represented a mortality rate of over 69% (9/13) in this flock of juvenile rheas, which might mean the infection is highly lethal in greater rheas (*Rhea americana*).

Wild species of birds are exposed and susceptible to avian poxviruses from other species. However, there is a certain degree of host specificity, and infections are not always as severe in certain species as they can be in the specific species targeted by one poxvirus strain.

Since there is no vaccine specifically made or approved for rheas, prevention and biosafety measures are the best ways to avoid this disease in wild and farmed individuals. Epidemiological factors that influence the pathogeny of avian pox in rheas need more research.

Given the outbreak presented in this study, contact with other domestic species of birds may have been relevant to the rhea's infection.

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**Conflict of interest statement.**- The authors declare that there are no conflicts of interest.

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