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Short communication

SYSTEMIC GRANULOMATOUS PATHOLOGY IN TWO CAPTIVE ALLIGATOR MISSISSIPPIENSIS

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The literature in this field cites various ubiquitous fungal and bacterial microorganisms as etiologic agents in severely stressed captive alligators and crocodiles. This study reports two cases of *Alligator mississippiensis* with bacterial and fungal disease. Two adult American alligators have been submitted for post-mortem investigations. Necropsy, cytology (MGG), and histopathology investigations (HE, HEA, PAS, Gram, Giemsa, Ziehl Neelsen) were carried out. Pleural and pericardial swabs were subjected to microbiological examination. The main lesions detected involved the lower respiratory system and were characterized by thoracic serosanguineous effusions, pleural and pulmonary nodules (1 – 80 mm), accompanied by edema. Similar nodules observed also in the liver, spleen and myocardium, suggested a systemic disease. Additionally, cutaneous, gingival and gastrointestinal erosions and ulcers were found. Cytoarchitecture findings in the major organs revealed lymphoid depletion, multifocal to coalescing necrotic areas with coccoid aggregates and rod shaped bacteria intermixing fungal structures, boarded by heterogeneous inflammatory infiltrates, composed by epithelioid macrophages, lymphocytes and heterophils. The microbiological examination revealed the presence of *Aeromonas hydrophila*, *A. caviae*, *Serratia marcescens*, *Pantoea agglomerans*, *Proteus vulgaris*, haemolitic and non-haemolitic *E. coli*, *Citrobacter freundii*, *Rhizopus/Absidia* from pleural and pericardial cavities, concluding that death occurred following a bacterial and fungal pneumonia, with secondary spreading of microorganisms. Along with the low immune response, severe stress was the main possible cause, as a result of environmental temperature changes during the winter, as well as other husbandry issues.

Key words: Alligator mississippiensis, systemic disease, histopathology, husbandry.

INTRODUCTION

According to the literature, numerous ubiquitous organisms, both bacterial and fungal, may become etiological agents for captive reptiles.

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Members of the order *Crocodylia* are ectotherms, which emphasizes – especially for the captive reptiles – the importance of temperature in the surrounding environment. Moreover, factors such as overcrowding, inadequate sanitation, chronic stress, improper nutrition, poor water quality, trauma, social stress, inadequate ventilation, improper substrate result in the release of corticosteroids and depression of the immune system [1-11]. On top of that, bacterial and fungal infectious agents from air, water, intestinal flora or food, may trigger severe infections, either local or septicemic. The most probable way for these infectious agents to reach the bloodstream is by escaping the intestine during severe stress conditions [7,8,12] although cases of septicemia caused by skin lesions have also been reported [2,5].

The current paper presents, for the first time in Romania, two cases of systemic pathology in *Alligator mississippiensis* that died because of a bacterial and fungal infection, during the winter, in a private facility.

MATERIALS AND METHODS

Two American alligators (*Alligator mississippiensis*), male and female, were registered and submitted for postmortem investigations at the Pathology Department of the Faculty of Veterinary Medicine in Bucharest, one in February and the other one in March, 2014. Necropsy, cytological examination (May Grunwald Giemsa) and histopathology (Hematoxylin Eosin, Hematoxylin Eosin Methyl Blue, Periodic Acid Schiff, Gram, Giemsa, Ziehl-Neelsen) were conducted. Pleural and pericardial samples were collected for microbiological examination.

RESULTS AND DISCUSSION

Grossly, both alligators exhibited erosions and ulcerations of the skin, with or without scale loss, on the ventral part of the body, including the mandible and the limbs. The lesions were either subacute or chronic. Paravertebrally, the female exhibited two penetrating lesions, approximately two cm wide. Both animals had multifocal to coalescing erosive and ulcerative lesions of the gingiva, covered with a yellow-greyish material – necrotizing stomatitis. The nostrils showed mucopurulent secretions. For both individuals, one eye was normal and the other presented structural changes with a gross aspect of atrophy. The cloacal orifice of the male revealed a yellowish foul odor discharge covering a 1cm/4cm yellow nodular lesion of the penis.

The peritoneal cavity of both alligators exhibited 100 ml of orange- color exudate. Similar effusions were found in the thoracic (100 ml) and pericardial (30 ml) cavities, the only difference being the fibrin deposit found floating in the pericardial cavity of the female. In addition, pleural adhesions were noticed, represented by white velutinous areas, corresponding to the pulmonary lesions.

Figure 1. **1** – Nasal mucopurulent discharge; **2** – Cutaneous erosions; **3** – Fibrinonecrotic stomatitis; **4** – Thoracic wall – pleural and pulmonary mycotic granulomas; Serosanguinolent effusions.

The lungs of both alligators were dark red, with multifocal to coalescing white oval areas, ranging from 1 mm to 8 cm in diameter. Sectioning of the lungs revealed a red inflammatory exudate, while the aforementioned lesions were cavitary, with white buttery deposits. The gross lesions suggest a granulomatous pneumonia.

The heart, for both reptiles, presented two white subepicardial foci, 1 and 2 mm wide, suggesting granulomatous myocarditis.

The digestive tract content was mostly represented by a small quantity of orange mucus and water, except for the stomach, in which wood, stone and glass fragments were found. The gastric and enteric mucosa was covered by multiple petechiae and erosions, suggesting acute gastritis and enteritis.

The liver of both alligators was dark red and revealed multiple discolored areas, less than 1 cm in diameter each, with a central white area, both observed under the capsule and within the hepatic parenchyma.

The spleen, dark red, exhibited a similar nodular aspect with white central areas.

The kidneys and genital organs (others than the male cloaca and penis) presented no gross pathology.

Figure 2. 5 – Multifocal to coalescing mycotic pleural and pulmonary granulomas; **6** – Multifocal to coalescing mycotic pulmonary granulomas; Edema; **7** – Myocardial granuloma; **8** – Granulomatous hepatitis

The adipose and muscular tissues were well represented.

Histopathology revealed that the thoracic wall was deeply affected in the areas corresponding to the pulmonary lesions. The muscular tissue was necrotic, oxyphilic and showed discrete hemorrhages and a moderate heterophilic and lymphocytic infiltrate, with intralesional fungal pseudohyphae.

Lungs lesions were severe, confirming the granulomatous pneumonia. Areas of edema, interstitial bacterial pneumonia, abundant heterophilic and mononuclear infiltrate, hyperemia, hemorrhages, intralesional fungal spores (phagocytized) and hyphae, necrotic debris and areas of fibrosis have been observed.

The liver exhibited hyperemia of the sinusoids, mononuclear and heterophilic infiltrate, hemosiderin within macrophages, and hepatocellular necrosis with intralesional fungal elements.

The most severe aspect in the pathology of the spleen was the lymphoid depletion, seen as a small number of cells within the white pulp, represented by small, inactive lymphocytes, rare plasma cells and few large lymphocytes. Occasional apoptotic lymphocytes were also observed. The red pulp was well represented, with hyperemia and hemosiderin within macrophages.

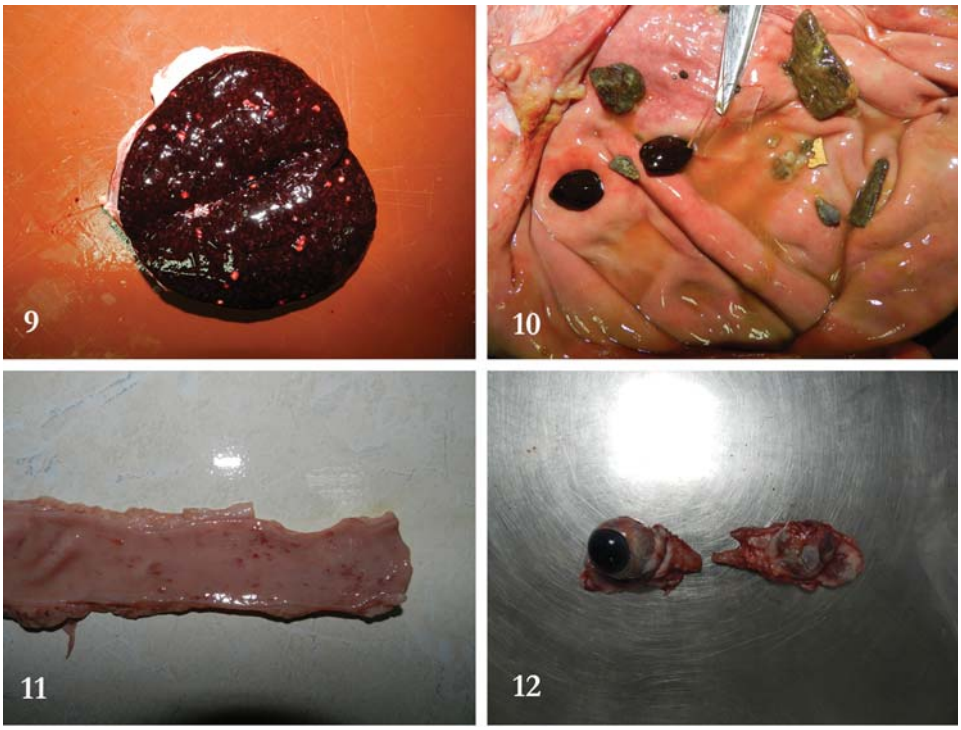

Figure 3. 9 – Granulomatous splenitis; **10** – Glass, wood, stone fragments; Gastritis; **11** – Intestinal petechiae; **12** – Unilateral eye atrophy

Microscopic examination of the kidneys revealed a membranous glomerulitis, with necrotized renal cells, desquamation of the tubular epithelium and mixed inflammatory infiltrate, together with hyperemia.

The microbiological examination yielded *Aeromonas hydrophila, Aeromonas caviae, Serratia marcescens, Pantoea agglomerans, Proteus vulgaris*, haemolitic and non-haemolitic *E. coli*, *Citrobacter freundii, Rhizopus/Absidia* from the pleural and pericardial cavities.

In most of the reported cases, the mycoses of the alligators are cutaneous and pulmonary, although organs such as liver or intestine have been occasionally involved [1,2,6,8,13-15].

As in this case, most fungal infectious agents are opportunistic, ubiquitous, being usually found in soil, water or plants, and are normally found in the intestinal flora [2]. Poor husbandry and severe stress lead to escaping fungi from the intestine into the blood flow. The physiologic bacterial flora of the intestine can inhibit overgrowth of fungi. However, a suppressed immune system, or a prolonged therapy with antimicrobial agents, can break the balance and predispose to fungal septicemia. The host's tissue reaction to fungal agents is usually granulomatous [7,8]. Species such as *Fusarium solani, Fusarium moniliforme, Fusarium verticillioides, Aspergillus ustus, Aspergillus fumigatus, Beauveria bassiana, Acremonium* (formerly *Cephalosporium*), *Candida albicans, Metarhizium spp., Mucor spp., Paecilomyces farinosus, Paecilomyces lilacinus, Penicillium spp., Stachybotrys spp., Chaetomium spp., Mucor spp., Rhizopus spp.* have been linked to infections in crocodiles [1,6,11,13-20].

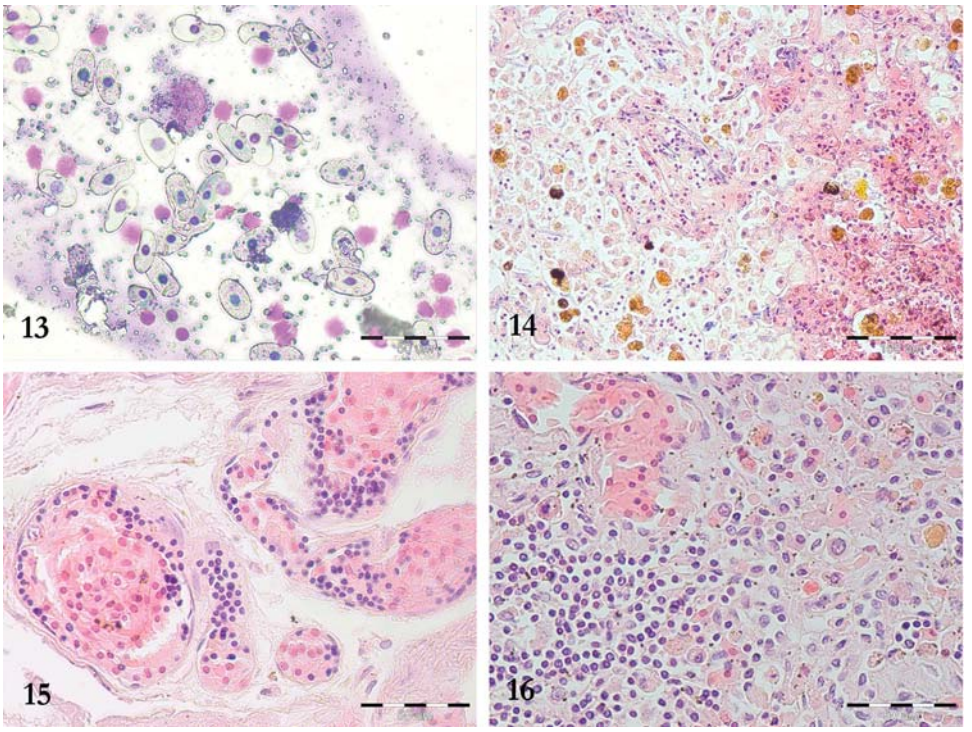

Figure 4. 13 – Cytology – Lung –Macrophages with phagocitized spores - MGG – 400x; **14** – Liver – Mycotic spores, dilated bile ducts, necrosis – HE - 200x; **15** – Lung – Mild inflammatory infiltrate - HE - 400x; **16** – Spleen – Lymphoid depletion - HE - 400x

In most cases, suboptimal temperatures were linked to the morbidity of the animals. Studies show that when the temperature decreases below the optimal value for these species, a depression of the metabolism and physiological functions occurs [11]. The ability of the fungi to multiply even at low temperatures, added to other stress factors as improper nutrition and husbandry, lead to an increased level of seric corticosterone, causing a depression of the immune system [3,4,21].

The same mechanisms and principles apply for the bacterial agents that are responsible for the morbidity and mortality. Bacterial species such as *Aeromonas hydrophila, Aeromonas shigelloides, Citrobacter freundii, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pasteurella multocida, Morganella morganii, Serratia marcescens, Serratia liquefaciens, Serratia fonticola, Salmonella spp., Shigella spp., Pseudomonas spp., Streptococcus spp., Bacillus spp., Clostridium spp., Actinomyces spp., Enterobacter agglomerans, Kiebsiella oxytoca, Morganella morganii, Providencia rettgeri, Mycobacterium spp., Mycoplasma alligatoris, Erysipelothrix insidiosa, Edwardriella spp.* are the ones that have been cited to produce organ or systemic infections in captive alligators or crocodiles [1,5,6,8,10,11,16,19,22-26]. Many of them have been reported producing lesions of the skin or of the lungs, and finally being incriminated in developing septicemia and lead to the death of the animal. The wide majority of the animals suffered from different types of stress, they were of different ages and have been captive in farms, circuses or zoos [2,12,27].

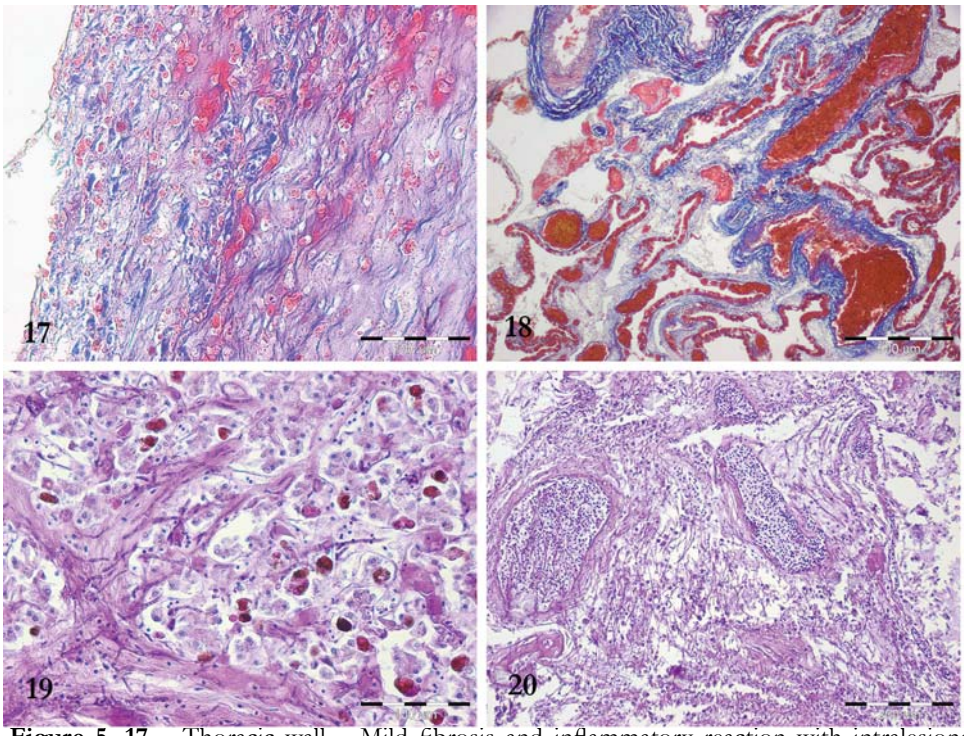

Figure 5. 17 – Thoracic wall – Mild fibrosis and inflammatory reaction with intralesional fungal hyphae - HEA – 200x; **18** – Lung - Fibrosis - HEA - 40x; **19** – Liver – Intralesional fungal hyphae - PAS - 200x; **20** – Lung – Intralesional fungal hyphae and mild inflammatory infiltrate - PAS - 100x

In both alligators, wood, stone and glass fragments were found in the stomach, in the absence of food, causing erosions. There are a few theories concerning foreign bodies in the digestive tract of alligators. The first one suggests the accidental swallowing of these fragments, alone or together with the food [8]. Another theory is that the fragments are used to grind the food, just like a gizzard in birds [28]. However, this theory is not supported by the literature in this field. Studies show that alligators don't need stones for digestion, just as carnivorous birds don't use stones in their gizzards [8, 29].

Although the high frequency of gastroliths in crocodiles may even be regarded as normal, another hypothesis is that it is due to an abnormal behavior caused by stress. Huchzermeyer describes three types of behavioral disturbances: anorexia, hydrophobia and excessive litophagy [8]. The last one does not only involve stones as many other foreign materials can be actively swallowed, such as wood, coal, nuts, nails, shotgun shells, dog tags, sinkers, fishing lines [30], toothpicks [31], coins [32], wire mesh [8], Coca Cola bottles [33]. There is another category of materials that, although part of the feed, can act as a foreign body: bezoars. Trichobezoars were found in crocodiles fed with rats, while a crocodile in poor condition was found with a distended stomach,

due to large wing feathers [8]. A common crocodile hunting method in Africa is to use string tied sticks embedded into fish or frogs and use it as bait. If the string breaks and the stick reaches the stomach, it may produce injuries [8] but it is not the case in the present paper. In the best case, the foreign objects can be regurgitated. This has been observed for hairballs or foreign objects in *Alligator mississippiensis* [8, 34]. If the animals are unable to regurgitate, morbidity appears, and it can be manifested in correlation with the shape and material of the foreign body. Sharp objects produce erosions or penetrate the stomach wall and other organs, and lead to peritonitis and necrosis. The alligator that ingested the coins was diagnosed with hepatitis and cirrhosis due to metal toxicity [32].

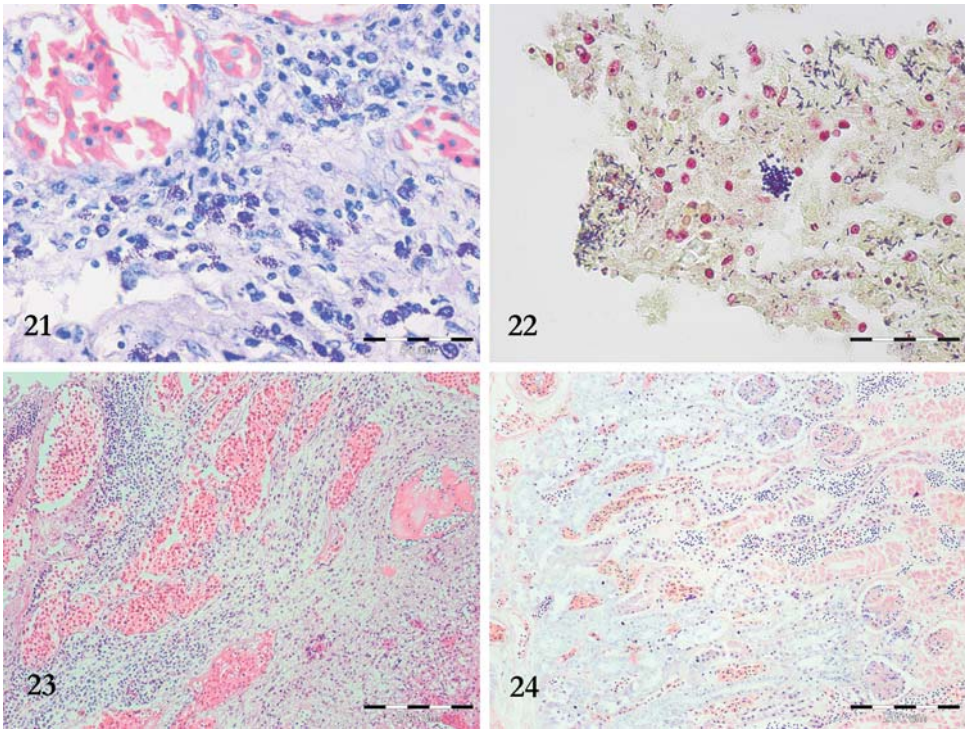

Figure 6. 21 – Lung – Phagocytized coccoid bacteria and inflammatory reaction - GIEMSA - 400x; **22** – Lung – Intralesional GRAM positive and negative bacteria with mycotic spores - GRAM - 400x; **23** – Lung – Interstitial pneumonia - HE – 100x; **24** – Kidney – Interstitial nephritis - HE – 100x

The outline of our study is that, in order to succeed in keeping, breeding or farming these kinds of reptiles, one must reproduce the conditions of the wild habitat as strictly as possible. This includes temperature, density of the animals, nutrition, air, water and substrate quality, and many more factors that will ensure the homeostasis and health of the animals. Studies argue the idea that, once infection occurs, medication alone may not be sufficient to cure the animal without removing the stressors [2,

8]. The death of the alligators was caused by the bacterial and fungal pneumonia, with secondary spreading of microorganisms, which developed a systemic disease. Although no investigations were carried out within the habitat of these animals, it is safe to assume that the death of the alligators was linked to the husbandry parameters that could not compensate the winter season properly.

Authors' contributions

RIR participated in the necropsy, gross diagnosis, histopathologic diagnosis, managed the sending of the microbiology samples, design of the study, draft the manuscript, given final approval of the version to be published and agree to be accountable for all aspects of the work. ST participated in the necropsy, gross diagnosis, histopathologic diagnosis, given final approval of the version to be published and agree to be accountable for all aspects of the work. ECP participated in the necropsy, gross diagnosis, given final approval of the version to be published and agree to be accountable for all aspects of the work. MM participated in the histopathologic diagnosis, design of the study, revising and coordination of the study, given final approval of the version to be published and agree to be accountable for all aspects of the work.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SISTEMSKA GRANULOMATOZA KOD DVA *ALIGATORA MISSISSIPPIENSIS* DRŽANIH U ZATOČENIŠTVU

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Literatura u ovoj oblasti navodi niz ubikvitarnih gljivičnih i bakterijskih mikroorganizama kao osnovne etiološke faktore bolesti krokodila i aligatora u zatočeništvu. Ova studija navodi prikaz dva slučaja bakterijske i gljivične etiologije kod *Alligator mississippiensis* u zatočeništvu. Urađena je postmortalna pretraga dva odrasla američka aligatora. Izvršene su: obdukcija, citološka (MGG) i histopatološka ispitivanja (HE, PAS, HEA, Gram, Giemsa i Ziehl Neelsen). Mikrobiološka ispitivanja su izvršena na brisevima pleure i perikarda. Glavne lezije su uočene na donjim respiratornim putevima i karakterišu ih serohemoragični eksudat, subpleuralni i granulomi u plućima (1-80 mm) kao i edemi. Slični čvorići su uočeni u jetri, slezini i miokardu što navodi na zaključak

da je u pitanju sistemsko oboljenje. Pored navedenih, uočene su i kutane, gingivalne i gastrointestinalne erozije.

Histološki nalaz organa je otkrio limfoidnu depleciju, multifokalna i nekrotična polja sa kokoidnim agregatima i štapićastim bakterijama koje se prepliću sa gljivičnim strukturama, ograničenim heterogenim inflamatornim infiltratom koji se sastoji od epiteloidnih ćelija - makrofaga, limfocita i heterofilnih granulocita. Mikrobiološki nalaz navodi prisustvo *Aeromonas hydrophila, A. caviae, Serratia marcescens, Pantoea agglomerans, Proteus vulgaris*, hemolitičke i nehemolitičke *E. coli, Citrobacter freundii, Rhizopus/Absidia* u perikardu i pleuri, što navodi na zaključak da je uginuće nastalo kao posledica bakterijske i gljivične pneumonije uz sekundarno širenje mikroorganizama. Uz slab imunski odgovor, stres usled temperaturnih kolebanja u zimskom periodu, kao i ostali ambijentalni uslovi uticali su na razvoj bolesti.