

Case Report / *Olgu Sunumu*

Pathologic findings of acantholytic squamous cell carcinoma coexisting with cutaneous cryptococcosis in a *Houbara bustard* (*Chlamydotis undulata*)

Reza KHEIRANDISH^{1,a}, Soodeh ALIDADI^{2,b}, Shahrzad AZIZI^{1,c,✉}, Atena AZAMI^{3,d}

¹Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran; ²Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; ³Department of Pathology, Imam Khomeini Hospital, Ilam University of Medical Sciences, Ilam, Iran

^a ORCID: 0000-0003-2540-3415; ^b ORCID: 0000-0003-2341-8533; ^c ORCID: 0000-0003-3746-9593; ^d ORCID: 0000-0001-5561-7575

✉Corresponding author: azizi@uk.ac.ir

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Abstract: A 2-year-old Asian *Houbara bustard* was presented with a solitary well-defined, firm cutaneous mass on the hock region. Grossly, the mass protruded from the surface was located on the hairless and unpigmented areas of the right hock joint with ulceration and dried hemorrhagic foci. On microscopic examination, ulceration, hemorrhage, as well as hyperkeratosis were observed. Large round, oval to polygonal neoplastic cells extended into the dermis were arranged to form cords, trabeculae, islands or glandular-like structures without keratin pearls. These pseudoglandular structures were composed of pseudolumina containing acantholytic and detached tumor cells. Necrosis of the neoplastic cells was accompanied by infiltration of inflammatory cells particularly heterophils. Unlike pleomorphic tumor cells, mitosis count was almost frequent. No evidence of other abnormalities and tumor metastasis was found. These gross and microscopic features appeared to be suggestive of a rare histologic variant of squamous cell carcinoma (SCC), acantholytic SCC.

Keywords: Acantholytic squamous cell carcinoma, *Chlamydotis undulata*, histopathology, *Houbara bustard*.

Squamous cell carcinoma (SCC) is one of the most common malignant skin tumors in all species of domestic animals. It occurs most commonly in dogs, cats, cattle, horses, less commonly in sheep, goats, and rarely in chickens (4, 7). Histopathologically, several subtypes of SCC are identified including well-differentiated, poorly differentiated, spindle cell, acantholytic and verrucous SCC (7, 15, 16). Acantholytic SCC, also known as adenoid or pseudoglandular SCC, is an uncommon intermediate- to high-risk subtype of SCC (15, 16). In addition to the skin, this uncommon subtype of SCC has been reported in non-dermal locations including the oral cavity, tonsil, maxilla, palate, esophagus, cecum, nasal cavity, lung, uterine cervix and vulva as well as breast. In the veterinary literature, there are only a few reported cases of acantholytic SCC in animals. It has not been previously reported in poultry unlike scattered reports of acantholytic SCC in dogs (4, 8, 21, 23) and cattle (12).

Cryptococcosis is a fungal infection mainly caused by *Cryptococcus neoformans*. This opportunistic pathogen affects immunocompromised hosts and invades the central nervous system and lungs (6, 9). Skin is the third most common tissue that may be infected. Cutaneous infection occurs third most common clinical manifestation of cryptococcosis. It is associated with the excreta of certain birds, including pigeons, canaries, and cockatoos (5).

The present study describes pathologic features of concurrent cryptococcosis and acantholytic SCC in a rare bird, Asian *Houbara bustard* (*Chlamydotis undulata*).

An approximately 2-year-old Asian *Houbara bustard* was referred to a veterinary hospital with a large solitary mass on the region of the hock joint. Grossly, the skin mass located on the right hock joint was sessile, firm, round to cauliflower-like, well circumscribed but non-encapsulated protruding from the surface (Figure 1). In addition, it measured three cm in diameter and the surface of the mass was slightly ulcerated with dried hemorrhagic

foci in some regions without secondary bacterial infections. In palpation, the mass had a firm consistency with a rough surface and gray to dark brown on cut section. The affected skin was unpigmented and hairless, and the mass did not invade the underlying bone and remained intact. No other abnormalities, tumor invasion and metastasis were found in necropsy. After removing the mass, taken samples were fixed in 10 % neutral buffered formalin (NBF) and then processed for paraffin embedding after fixation. The samples were sectioned at 5 μ m and stained with hematoxylin-eosin (H&E). The number of mitotic figures was counted in 10 contiguous fields, with no overlapping, high-power field (HPF). Mitotic counts per field were calculated as:

$$\text{Mitotic count/field} = \frac{\text{total number of mitotic figures observed}}{\text{number of fields counted}}$$

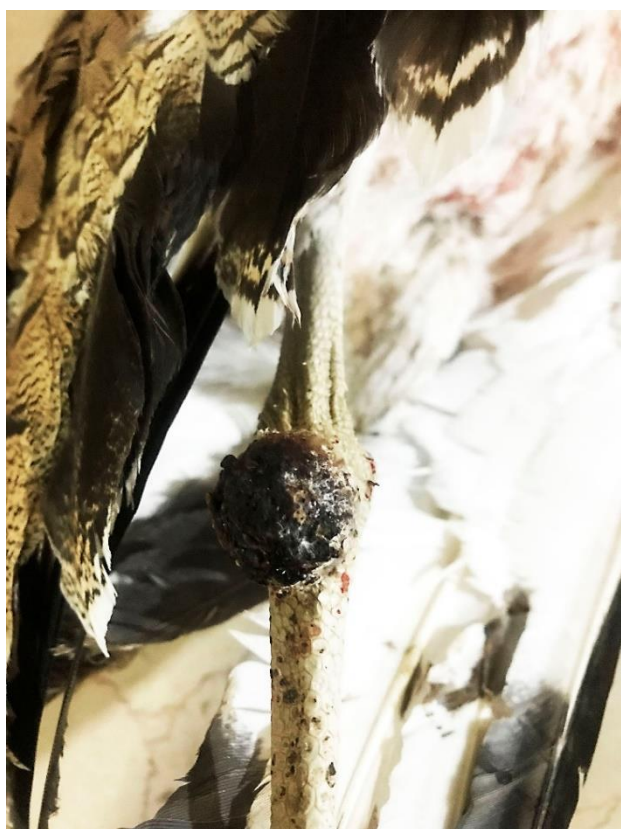


Figure 1. Right leg showing a large solitary skin mass attached to the hock joint in *Houbara bustard*. Hemorrhagic surface of firm, well-defined but non-capsulated mass with no secretion is evident.

Histologic examination revealed hyperkeratosis and parakeratosis over the epidermis surface of the mass. Moreover, the epidermal surface of the mass was ulcerated and hemorrhagic in some areas (Figure 2A). The tumor consisted of large round and oval to polygonal cells arranged in cords, trabeculae or islands extended into the underlying dermis. No evidence of intracytoplasmic eosinophilic keratin tonofilaments was observed and there were no keratin pearls characteristic of well-differentiated

SCC. Interestingly, some neoplastic keratinocytes underwent dyskeratosis or filamentous degeneration. More importantly, there was marked dyshesion of the non-delimited neoplastic cells so that basal cells were the only cells attached to the basal lamina in some sections (Figure 2B, 2C). The gland-like or pseudoglandular structures were comprised of tumor islands with the cohesive outer layer and floating individual and/or clustered acantholytic or/and dyskeratotic keratinocytes in the center of false lumina (Figure 2C, 2D). In some areas of the tumor, numerous cells underwent extensive necrosis and the necrotic keratinocytes were infiltrated by inflammatory cells (Figure 3A). Inflammatory cells particularly heterophils were visible around the necrotic areas to phagocytose the dead cells and tissue remnants (Figure. 3B, 3C). The neoplastic cells had pale to brightly eosinophilic cytoplasm with a large round to ovoid vesicular nuclei containing loose chromatin. Some neoplastic cells had more than one prominent nucleoli (Figure 3C). Mitotic figures were frequent and different phases of the mitosis were observed (Figure 3D). Conversely, there was not considerable cellular and nuclear pleomorphism. In accordance with macroscopic and microscopic features, a diagnosis of acantholytic or pseudoglandular SCC was provided. PAS staining of tissue samples showed a lot of oval to spherical-shaped microorganisms with positive mucinous capsule in areas of proliferated squamous cells and also in the necrotic debris of pseudoglandular structures of the tumor (Figure 4). Based on morphology, *Cryptococcus neoformans* infection was diagnosed.

Acantholytic SCC is an uncommon histologic variant of SCC in which neoplastic cells form pseudoglandular structures with variably sized discohesive acantholytic cells (15). The histopathologic characteristics of the neoplasm in the present case are extremely consistent with those observed in the medical and veterinary literature (21, 23). The pathogenesis and precise mechanism responsible for the development of acantholytic SCC have not been entirely understood. However, it has been demonstrated that decreased expression or loss of at least one desmosomal protein such as the cadherin family and subsequent loss of cell-cell adhesions potentially contribute to 89 % of the pseudoglandular structures formed in acantholytic SCC. Likewise, it has been illustrated that two or more desmosomal proteins responsible for tissue cohesion have been lost in 65 % of the acantholytic SCC (10). On the other hand, although acantholytic SCC might arise at any site in different species, several factors can significantly influence the tumor development including overexposure to sunlight and ultraviolet light of hairless and lightly pigmented sites (15). As a result, receiving a large load of ultraviolet radiation can also be suggested as one of the probable etiologies for acantholytic SCC.

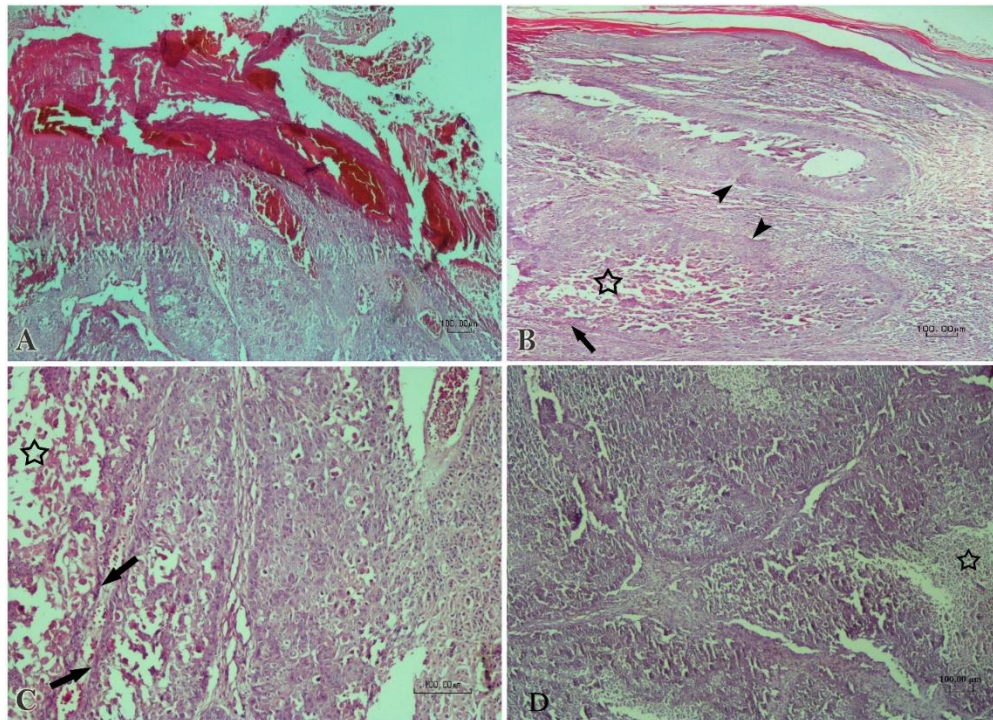


Figure 2. Acantholytic squamous cell carcinoma.

A. Occurrence of ulceration and hemorrhage on the epidermal surface of tumor. Islands of neoplastic squamous cells are typically extended into the dermis.

B. Mild hyperkeratosis with ulceration of the epidermis in the left side of the illustration. Pseudoglandular structures (arrowheads) with neoplastic keratinocytes (arrow) are obvious in the dermis. Some neoplastic cells are sloughed (empty star).

C and D. A higher magnification of glandular-like structures with clustered acantholytic keratinocytes floated in the lumen (empty star) so that the basal layer is the only remained layer (arrows). H&E staining. Bar = 100 μ m.

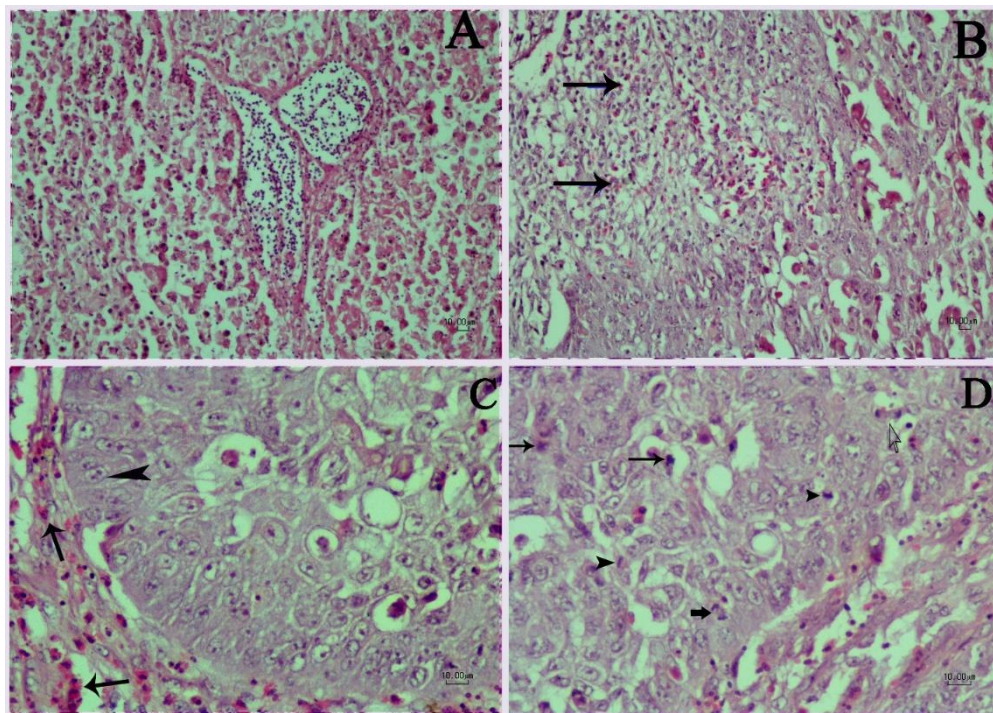


Figure 3. Acantholytic squamous cell carcinoma.

A. Extensive dermal degeneration and necrosis of the neoplastic cells.

B. Infiltration of heterophils in the stromal tumor (arrows).

C. Neoplastic cells with vesicular nuclei and prominent nucleoli (arrowhead) and also heterophils infiltration in the stroma (arrows)

D. Different stages of mitotic figures including prophase (arrows), metaphase (arrowheads) and anaphase (empty arrow). H&E staining. Bar = 10 μ m.

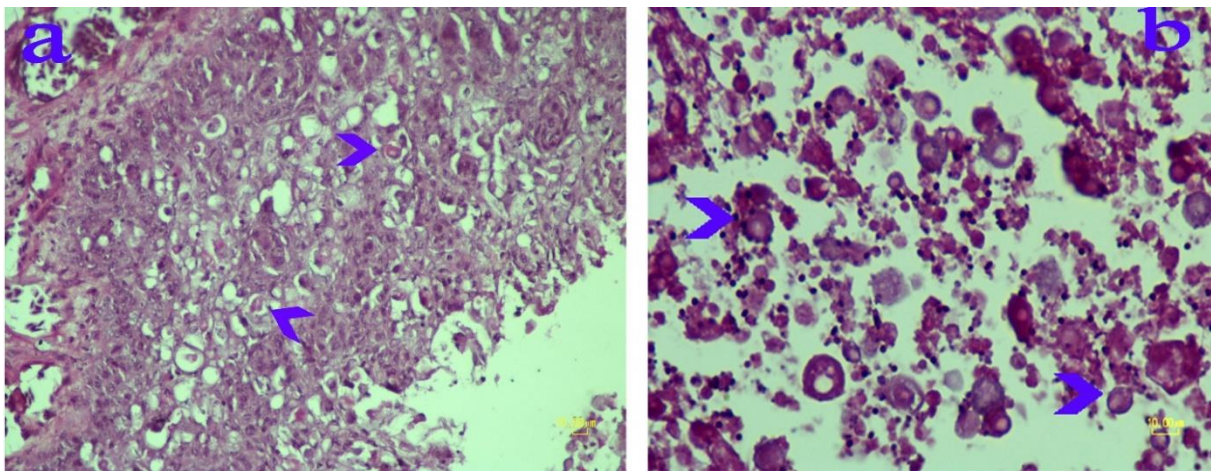


Figure 4. Periodic acid–Schiff (PAS) staining shows positive mucinous capsule of *Cryptococcus neoformans* (arrowheads) between proliferated squamous cells (a) and necrotic debris in the tumor (b).

Although the aggressiveness and malignancy of acantholytic SCC compared with conventional SCC are debated in the literature (8, 11, 23), the former has been proposed to display a more aggressive clinical behavior on the basis of the literature review conducted by Mohammad and Wilcox (16). Due to rarity of acantholytic SCC and low numbers of these cases, the biologic and aggressive behavior of the tumor has not been precisely determined in the literature. In accordance with the present research, three cases of acantholytic SCC reported in animals have not showed any evidence of metastasis (4, 8, 12). Nonetheless, metastasis to regional lymph nodes and manifestation of considerable clinical signs has been reported in 2 cases of the neoplasm (21, 23). Romanucci et al. (21) reported acantholytic SCC in the external ear canal of dogs with metastasis to the submandibular, parotid, retropharyngeal, cervical, prescapular lymph nodes and the lung. In another study, the tumor in the submandibular region had metastasis to the cervical and submandibular lymph nodes (11). Nevertheless, the prognosis of acantholytic SCC varies depending on the location size, and grade of the tumor as well as the host characteristics (10). The tumor location and its proximity to the regional lymph nodes are counted as critical factors influencing the tumor aggressive behavior. The tumor was well-demarcated from the surrounding tissues and the underlying joint and bones in the present research. Consequently, it did not show aggressive behavior and extension to the adjacent tissues. Nevertheless, mitotic figures were present (3-4 mitoses/400× field) and the frequency of mitotic figures might increase with the degree of malignancy (7).

As mentioned previously, there are few reports on the occurrence of acantholytic SCC in animals such as dogs and cattle to date, but here we reported this variant of SCC in a rare bird. The *Houbara bustard*, *Chlamydotis*

undulata macqueenii, is a rare ground-dwelling steppe bird that inhabiting in desert and semi-desert areas (25). Thereby, the exposure to the strong and direct sunlight might be a possible risk factor for the neoplasm development in the hairless and unpigmented site of the hock region of the *Houbara* in our study. In the present case, concurrent cutaneous cryptococcosis (PCC) infection was observed. Cryptococcosis is an opportunistic fungi disease found throughout the world. PCC occurs as primary and secondary forms. Primary form mainly results from direct skin trauma and aberrations such as, papules, nodules, tumors, ulcerations, ecchymoses, pustules, abscesses and granulomas (3, 13, 17). Secondary PCC a more common type spreads from the brain, lung and other parts of the body. Immunodeficient patients such AIDS, malignant tumors, organ transplantation, anti-neoplastic and corticosteroid therapy and immunosuppressive drugs are the most susceptible cohort for cutaneous cryptococcosis (18).

Cutaneous cryptococcosis usually becomes visible as fluctuant or firm nodules that some of them may be ulcerated. Direct inoculation of organisms into the skin occasionally leads to the formation of solitary lesions (20).

Mallany et al. (2021) described laryngeal cryptococcus infections in an 83-year-old man with a history of chronic asthma and chronic obstructive pulmonary disease (COPD). Histopathologic results showed atypical squamous proliferation, acute inflammation, and scattered round-shaped microorganisms surrounded by clear halos. It was diagnosed as laryngeal squamous cell carcinoma (SCC), due to an overlying laryngeal squamous cell proliferation (14).

Squamous pseudoepitheliomatous hyperplasia (PEH) is a reactive epithelial proliferation that may occur secondary to different factors including infection, injury and inflammation. PEH in relation to fungal infection may

provide a potential mechanism for the overlying squamous cell carcinoma (SCC) mimicry (19).

Infections in birds are rare. The bird showed patchy feather loss, especially around the back and beak area. The feathers had a greasy appearance and disseminated a moldy odor (24). Bird faces particularly from pigeon is an environmental reservoir for *C. neoformans*. This yeast is present in decaying trees, wood, soil and waterways that contaminated with bird excrement (1, 2, 22).

In the present study, except for sunlight, cryptococcus infection can be a risk factor for acantholytic squamous cell carcinoma formation in this bird. Further investigation is needed until the pathogenesis of this tumor be illustrated in wild birds.

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Ethical Statement

The current study is not an experimental part on living animals. Therefore, any approval from the ethic committee was not required.

Conflict of Interest

The authors declared no potential conflicts of interest.

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