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ORIGINAL ARTICLE

Anatomopathological aspects of avian aspergillosis

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Abstract Aspergillosis is a fungal disease caused by fungi of the genus *Aspergillus*, in particular *A. fumigatus* and *A. flavus.* This paper focuses on anatomopathological aspects resulting from a chronic infection from *Aspergillus* spp in the chicken (*Gallus domesticus*), in the herring gull (*Larus cachinnans micaelli*) and in the red-legged partridge (*Alectoris rufa rufa*). Microscopically, we observed some histological lesions that are related to the two typical forms of Aspergillosis: a deep nodular form, typical of organs with a non-aerated parenchyma, and a non-encapsulated superficial diffuse form typical of the serosae and the lung. The observed forms of aspergillosis have been found in animals raised in poor hygienic environmental conditions or malnourished animals (chicken); in wild birds from wildlife recovery centres (herring gull), which underwent some forms of stress, such as traumas, detention, starvation, extended antibiotic treatments; in game birds (red-legged partridge) used for restocking natural areas that had been negatively affected by such stressors as captivity in aviaries, containment and transport in cages, release in unsuitable environments and malnutrition. The observed anatomopathological and istopathological aspects can therefore be regarded as the outcome of a number of factors that have reduced the typical resistance of the species and impaired the efficiency of their immune systems.

Keywords Aspergillosis · Birds · Pathological findings

Background

Aspergillosis is a fungal disease caused by fungi of the genus Aspergillus, in particular *A. fumigatus* and *A. flavus*; it has been described in many farm and wild bird species, both in the natural and captive state (Tell 2005; Guillot and Chermette 2001). In poultry, a severe acute form has been observed mostly in young subjects, with high morbidity and mortality rates, and a chronic form in adult subjects, especially in turkey breeders (Martin et al. 2007; Radkowski et al. 1996). A similar epidemiology has also been observed in parrots, where

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acute forms in the young subjects, often associated with immunosuppressive viruses 1 (Simpson and Euden 1991), are combined with chronic forms, which diagnosis can be supported by radiography or endoscopy (Forbes et al. 1992).

Fungi of the genus Aspergillus are ubiquitous: they can be found in the soil, in feeds as grains, and decomposing vegetables (Perdelli et al. 2006). The dust from litters and feeds contains the spores, which, through this carrier, are scattered in the environment. The spores penetrate into the aerial apparatus, where they find the ideal conditions to germinate, produce vegetative forms and invade the tissues. The target organs are the lungs and the air sacs, but in their systemic forms they can also affect the liver, the kidney, the encephalon, the bones, the skin and the eyes (Raja et al. 2006; Trullas et al. 2005).

Histopathologically, the lesions are characterized by two different patterns: a granulomatous form with internally-septate hyphae, that typically develops into non aerate parenchyma, and a non-encapsulated diffuse forms, containing sporangia with a typical morphology, located in the lung or, even more frequently, in the air sacs (Nardoni et al. 2006).

This paper focuses on the different kinds of lesions that have been observed in farm and wild animals, all resulting from a chronic infection from *Aspergillus* spp.

Materials and methods

Examined animals belonging to the Laboratory of Avian Pathology - Department of Animal Pathology - of the University of Pisa; a group of 9 birds were examined, composed by:

- 3–4 months old - chickens (*Gallus domesticus*) belonging to a country farm;
- 5 herring gulls (*Larus cachinnans micaelli*), which had been admitted to and had died at the LIPU-CRUMA wildlife recovery centre in Livorno (Tuscany, Italy);
- 1 red-legged partridge (*Alectoris rufa rufa*) from an intensive breeding farm, released into the natural environment and enrolled in a study for the observation of their behaviour and adaptive skills.

These animals underwent a thorough post-mortem examination. The sampled pathological material was subjected to laboratory tests, in the attempt to find out, through appropriate culture tests, if they contained any fungi and/or bacteria.

Tissues with lesions suggestive of fungal infection, were removed and fixed in 10% buffered formalin, then paraffin-wax embedded for histological examination. 3 μm-thick sections were mounted on slides and stained with haematoxylin-eosin for a general histological view. Serial sections were also stained by Grocott's and Periodical Acid Shiff dye (PAS) to highlight any fungal hyphae.

A fresh portion of the same fixed-pathological material was also sowed in some media, such as Malt Extract Agar (MEA) and Sabouraud Dextrose Sugar (SDA), which are typical for fungi, and incubated at 25°C and 37°C for 4 to 10 days. Portions of the same damaged organs were homogenised and sowed in Blood Agar to detect a possible bacterial co-infection.

Results

The histological test detected some microscopic alterations that occurred in the damaged tissues, suggestive of aspergillosis, and the presence of fungal hyphas.

The post-mortem test showed different kinds of lesions in different sites. In the cockerels, diffuse granulomatous lesions were observed in the air sacs, involving parietal and visceral serosae too; they ranged from miliary to large granulomatous foci, white in colour, dry in texture and protruding out of the surface of the affected organ (Fig. 1).

In the partridge, brownish patches were observed on the surface of the parietal serosae and similar lesions were detected deep into the pulmonary parenchyma. They looked compact, with a neatly cut cross-section and a necrotic-caseous texture (Fig. 2).

In the herring gulls, large lesions were observed in the lungs and air sacs which looked completely covered in whitish caseous material, with a grey-greenish mould on it, which suggested fungal sporulation (Fig. 3).

The bacteriological and fungal tests detected *Aspergillus* spp in such lesions and excluded the concurrent presence of a bacterial infection.

The histological picture observed into un-aerate organs as liver and spleen consists of focal nodular formations which resemble tubercles. These tubercle-like nodules show in the centre a radiating turf of hyphae surrounded by a reactive inflammatory wall which resembled granulation tissue, with participation of foreign body giant cells.

The histological examination conducted on portions of the lungs, air sacs and serosae of the animals that had tested positive at the fungal culture, showed instead that such lesions were composed of caseous necrotic material surrounded by giant cells, macrophages, heterophils and lymphocytes. These "diffuse" forms differed by the absence of a very well structured tubercle-like granulomata formations around the hyphae, with or without septa, which were located inside such material and were clearly visible by means of the PAS dye. In particular, histology of the pulmonary lesions observed in the gulls showed a massive development of the vegetative forms of the fungus. The fruiting organs as conidiophores, sterigmata , and conidia, were observed only in the air sacs and lung's parenchyma (Fig. 4).

Discussions and conclusions

Avian aspergillosis is a fungal disease that has been described, as well as in the chicken and turkey, also in other species, such as geese, ducks, quails, ostriches, parrots, canaries, pigeons, penguins and starlings (Atasever and Gűműşsoy 2004).

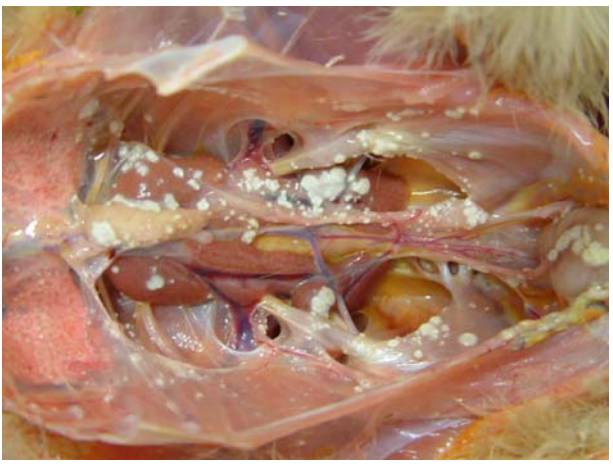

Fig. 1 Cockerels: diffuse lesions in the air sacs and on parietal and visceral serosae, with foci in varying size protruding from the surface of the affected organ, white in colour and dry in texture

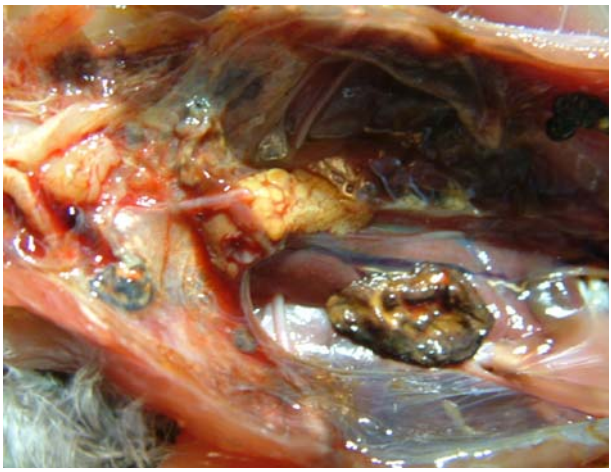

Fig. 2 Red-legged partridge: brownish patches, compact in appearance, with a clear-cut cross section and a necrotic-caseous texture on the surface of the parietal serosae and the pulmonary parenchyma

Macroscopically, the lesions we observed in the three species are substantially different in both morphology and location. Such histopathological differences are related to the two forms of the disease, the deep nodular form (typical of organs that have a non-aerated parenchyma) with a solid and well-organised granulomatous reaction, with more or less abundant inflammatory cells and a peripheral connective vallum variable in size, and the superficial diffuse form (typical of the serosae and the lung) with a massive development of the vegetative forms of the fungus, containing a large number of conidiophores, hyphas and spores. In the former case, we observed a neat, well-organised granulomatous reaction, with more or less abundant inflammatory cells, but above all with a peripheral connective vallum that widely varies in thickness. In the diffuse form, in addition to containing the fungus at a different biological stage, accordingly with other authors who observed the same lesions in several species (Beytut et al. 2004;) we observed a diffuse pyogranulomatous reaction, i.e. non-ridged, non-encapsulated, like a typical granuloma.

The lung is the most affected internal organ, as shown in the herring gull. We actually observed, in the lung of the herring gull, an extensive development of the vegetative forms of the fungus and the presence of a large number of conidiophores, hyphae and spores. In

Fig. 3 Herring gulls: lesions in the lungs and air sacs covered in a whitish caseous material, with grey-greenish mould on top, suggesting fungal sporulation

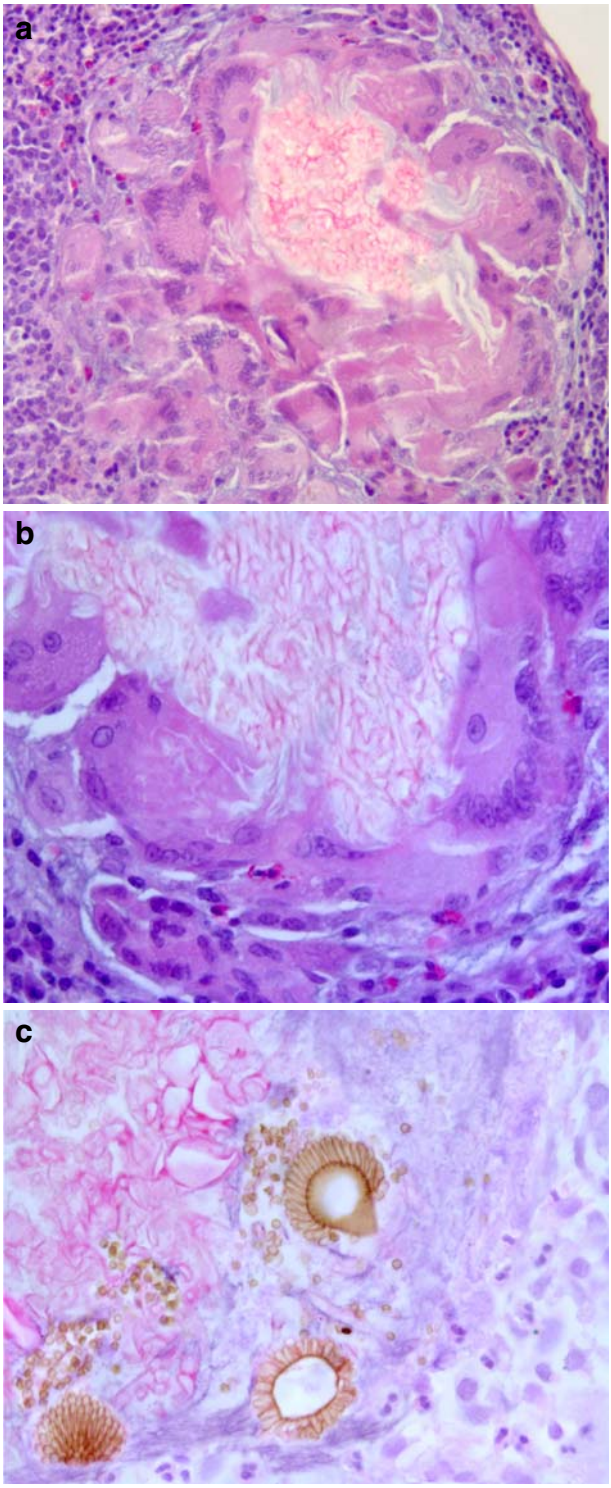

Fig. 4 a) Histological lesions with necrotic-caseous material surrounded by giant cells, macrophages, heterophiles and lymphocytes (40X); b) Fungal hyphas with or without septi are clearly visible inside, using the Periodical Acid Shift dye (PAS) (60X); c) Non-encapsulated diffuse pulmonary lesions, with a massive development of the vegetative forms containing sporangia with a typical morphology (60X)

this respect, according to Nardoni (Nardoni et al. 2006), many birds can host the spores of the *Aspergillus* spp in the lung and air sacs, leading to a dormant or chronic infection, with no clinical symptoms or apparent anatomopathological lesions.

In our case-studies, we observed three different clinical forms of the disease, with different anatomopathological patterns, related to two kinds of microscopic lesions, typical or organised and localised granulomatous lesions, and diffuse forms.

The full-blown, rapidly-killing clinical forms that we observed in cockerels from country or non-professional farms, generally, have been found in animals that were raised in malnourished animals, breeded in poor hygienic environmental conditions. In fact, birds are very susceptible to the Aspergillus infection (Lair-Fulleringer et al. 2003) and stressor as starvation, thermal and/or migratory stress, toxicosis adverse environmental conditions or trauma cause immunosuppression and allow to colonize tissues by opportunistic fungi (Richard and Thurston 1983; Ritchie et al. 1994; Akan et al. 2002; Carrasco et al. 2001).

The longest-developing forms have been observed in wild birds from wildlife recovery centres, especially following some stress, such as traumas, long detention in captivity, starvation, concurrent presence of other diseases, stress-induced immune depression and extensive antibiotic treatments.

Finally, in the partridge, we have assumed that the animal, raised in captivity, during and after released may have experienced some stressors, such as containment and transport in cages, an unsuitable new environment and the absence of it usually diet, allowed to more susceptibility to the Aspergillus infection.

In determining such widely different forms of the pathology, a key role is played by the typical resistance of the species, since immunodepression is one of the most important infection-inducing factors in all animal species (Singh et al. 1991; Deem 2003; Beernaert et al. 2008). The ability of a species to respond to the fungal antigens by organising a good and lasting cell-mediated response explains why, even with the same histological kind of lesion, some species respond by increasing the ridge and thickness of the granuloma's capsule, while others produce poorly defined lesions with a very small connective vallum and diffuse forms of cellular exudates.

Therefore the pathological forms of aspergillosis that we observed can be regarded as the outcome of a number of host-dependent factors, first and foremost the efficiency of the immune system of the host animal, which is responsible for fighting the systemic spreading of the infection (Lehmann 1985).

References

Akan M, Haziroglu R, Ilhan Z, Sareyyupoglu B. *A case of aspergillosis in a broiler breeder flock*. Avian Dis 2002; 49: 497–501. doi:10.1637/0005-2086(2002)046[0497:ACOAIA]2.0.CO;2

Atasever A., K. S. Gűműşsoy; *Pathological, clinical and mycological findings in experimental aspergillosis infections of starlings*. J. Vet. Med. A 2004; 51, 19–22. doi:10.1111/j.1439-0442.2004.00598.x

Beernaert LA, Pasmans F, Haesebrouck F, Martel A. *Modelling Aspergillus fumigatus infections in racing pigeons (Columba livia domestica)*. Avian Pathol 2008; Oct;37(5):545–9

Beytut E, Ozcan K, Erginsoy S. *Immunohistochemical detection of fungal elements in the tissues of goslings with pulmonary and systemic aspergillosis*. Acta Vet Hung 2004; 52(1):71–84. doi:10.1556/AVet.52.2004.1.8

Carrasco L, Lima JS Jr, Halfen DC, Salguero FJ, Sanchez-Cordon P, Becker G. *Systemic aspergillosis in an oiled magallanic penguin (Spheniscus magellanicus)*. J Vet Med B 2001; 48: 551–554. doi:10.1046/j.1439-0450.2001.00456.x

Deem SL. *Fungal diseases of birds of prey*. Vet Clin North Am Exot Anim Pract. 2003;May 6(2):363–76.

Forbes NA, Simpson GN, Goudswaard MF. *Diagnosis of avian aspergillosis and treatment with itraconazole*. Vet Rec. 1992; Jun 6;130 23:519–20.

Guillot J., Chermette R. *Aspergillosis in birds*. Rev Prat. 2001; Apr 15;51(7):704–7.

Lair-Fulleringer S, Guillot J, Desterke C, Seguin D, Warin S, Bezille A, Chermette R, Bretagne S. *Differentiation between isolates of Aspergillus fumigatus from breeding turkeys and their environment by genotyping with microsatellite markers*. J Clin Microbiol 2003; 41: 1798–1800. doi:10.1128/JCM.41.4.1798-1800.2003

Lehmann PF. *Immunology of fungal infections in animals*. Vet Immunol Immunopathol 1985; Oct;10(1):33–69.

Martin MP, Bouck KP, Helm J, Dykstra MJ, Wages DP, Barnes HJ. *Disseminated Aspergillus flavus infection in broiler breeder pullets*. Avian Dis. 2007; Jun;51(2):626–31.

Nardoni S, Ceccherelli R, Rossi G, Mancianti F. *Aspergillosis in Larus cachinnans micaellis: survey of eight cases*. Mycopathologia. 2006 May;161(5):317–21. doi:10.1007/s11046-006-0012-2

Perdelli F, Sartini M, Spagnolo AM, Dallera M, Lombardi R, Cristina ML. *A problem of hospital hygiene: the presence of aspergilli in hospital wards with different air-conditioning features*. Am J Infect Control. 2006 Jun;34(5):264–8. doi:10.1016/j.ajic.2005.12.004

Radkowski M, Uradziński J, Szteyn J. *The occurrence of infectious and parasitic diseases in poultry slaughtered in the district of Olsztyn, Poland, 1986-91*. Avian Dis. 1996 Apr–Jun; 40(2):285–9. doi:10.2307/1592222

Raja NS, Singh NN. *Disseminated invasive aspergillosis in an apparently immunocompetent host*. J Microbiol Immunol Infect. 2006 Feb;39(1):73–7

Richard JL, Thurston JR. *Rapid hematogenous dissemination of Aspergillus fumigatus and Aspergillus flavus spores in turkey poults following aerosol exposure*. Avian Dis 1983; 27(4): 1025–1033. doi:10.2307/1590203

Ritchie BW, Harrison GJ, Harrison LR. *Avian Medicine: Principles and Application*. Florida: Wingers Publishing, Lake Worth, 1994: 1384 pp.

Simpson VR, Euden PR. *Aspergillosis in parrots*. Vet Rec. 1991 Feb 23;128(8):191–2.

Singh N, Yu VL, Rihs JD. *Invasive aspergillosis in AIDS*. South Med J. 1991 Jul;84(7):822–7.

Tell LA. *Aspergillosis in mammals and birds: impact on veterinary medicine*. Med Mycol. 2005 May;43 Suppl 1:S71–3. doi:10.1080/13693780400020089

Trullas JC, Cervera C, Benito N, de la Bellacasa JP, Agustí C, Rovira M, Mas A, Navasa M, Cofan F, Ricart MJ, Pérez-Villa F, Moreno A. *Invasive pulmonary aspergillosis in solid organ and bone marrow transplant recipients*. Transplant Proc. 2005 Nov;37(9):4091–3. doi:10.1016/j.transproceed.2005.09.182