

OUTBREAK OF PSEUDOTUBERCULOSIS IN COMMERCIAL GUINEA FOWLS (*NUMIDA MELEAGRIS*)

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Abstract

The present paper reports an outbreak of pseudotuberculosis in guinea fowls reared for meat production. The clinical and pathological features as well as the results of the laboratory investigations are described. To the knowledge of the authors this is the first reported case of *Yersinia pseudotuberculosis* infection in guinea-fowls.

Key words: guinea fowl, septicaemia, *Yersinia pseudotuberculosis*.

Riassunto

Focolaio di pseudotubercolosi in faraone (*Numida meleagris*) d'allevamento

Il presente lavoro descrive un episodio di mortalità riconducibile ad una sindrome setticemica osservata in un gruppo di faraone da carne prossime alla macellazione. L'esame batteriologico ha permesso di isolare *Yersinia pseudotuberculosis* dai fegati e dalle milze di tutti i soggetti sottoposti ad autopsia. Tale microorganismo, che è stato isolato da ruminanti domestici e selvatici, suini, mammiferi ospiti di giardini zoologici e lagomorfi, sembra trovare nei roditori e negli uccelli selvatici il suo serbatoio naturale. In letteratura vi sono segnalazioni in volatili ornamentali di casi di pseudotubercolosi sia sporadici che epidemici e, tra le specie domestiche allevate intensivamente, sono segnalati focolai epidemici nel tacchino. Tuttavia non risulta alcuna segnalazione della malattia nella faraona. In considerazione del ruolo di serbatoio svolto da roditori e uccelli selvatici, i volatili d'allevamento, in particolar modo quelli che usufruiscono di parchetti esterni, sono potenzialmente esposti al rischio di infezione da *Y. pseudotuberculosis*. Pertanto in presenza di forme setticemiche, soprattutto caratterizzate da epatite necrotica, sarebbe opportuno porre in diagnosi differenziale anche la pseudotubercolosi.

Parole chiave: faraona, setticemia, *Yersinia pseudotuberculosis*.

Introduction

Yersinia pseudotuberculosis is a Gram negative, rod shaped, oxidase negative bacterium, member of the Enterobacteriaceae family. It has been isolated from wild and domestic ruminants, swines, mammals living in zoological gardens and hares even if rodents and wild birds are thought to be the main reservoir of this microorganism (Mair, 1973; Toma, 1986). *Y. pseudotuberculosis* can cause an acute septicaemia, with sudden death, as well as a less acute disease characterized by weakness, persistent diarrhoea, lameness, progressive emaciation and by the appearance of caseous tubercle-like lesions in parenchymatous organs. It can infect humans through ingestion of contaminated food and water and causes mesenteric lymphadenitis and acute terminal ileitis (Harcourt-Brown, 1978).

Surveys have been carried out in order to assess the prevalence of *Y. pseudotuberculosis* in wild birds and mammals through bacteriological examination of faecal or cloacal samples; they

revealed prevalences lower than 1% in birds and of 5.6% in a variety of mammals such as deers, hares, martens and racoon dogs (Levrè *et al.*, 1989; Fukoshima and Gumyoda, 1991; Niskanen *et al.*, 2003).

Sporadic and epidemic cases of pseudotuberculosis have been reported in ornamental birds; epatomegaly and splenomegaly, small grey-white nodules in the liver and spleen, and congestion of the lungs, were the most commonly observed lesions (Harcourt-Brown, 1978). Reports of the disease in domestic birds are rare and mainly concern turkey flocks. In the outbreaks described by Wallner-Pendleton and Cooper (1982) two kind of gross lesions were observed: enlarged livers and spleens with miliary grayish-white necrotic foci and catarrhal enteritis or enlargement and greenish discoloration of the livers and osteomyelitis (Wallner-Pendleton and Cooper, 1982). *Y. pseudotuberculosis* was isolated from the liver, the spleen and the bone marrow. Control of the disease was achieved by in-feed administration of tetracycline, but high condemnation rate was recorded at slaughter because of septicaemic lesions. Ground squirrels affected by pseudotuberculosis were identified as source of the infection in one of the outbreaks (Wallner-Pendleton and Cooper, 1982).

The present paper reports the results of the laboratory investigations carried out in a commercial guinea fowl flock affected by pseudotuberculosis. To our knowledge this is the first report of pseudotuberculosis in guinea fowls.

Material and methods

The carcasses of seven guinea fowls from a commercial farm were submitted to our laboratory on two different occasions, with a seven-day interval, for post-mortem investigations. In addition two birds were submitted alive, housed in our facilities and observed for two months in order to evaluate the progression of the illness. At death they were examined for gross lesions. At necropsy liver and spleen samples were collected to perform bacteriological examination. The samples were plated on blood agar and Eosin-Methylene blue agar. The plates were then incubated at $37\pm 1^{\circ}\text{C}$ for 24-48h under aerobic conditions. The identification of isolated strains was achieved by miniaturized biochemical test (API 20 E BioMerieux). Antimicrobial susceptibility testing was performed according to Kirby Bauer method using a standard panel of antibiotics for Gram negative bacteria. Isolation of *Salmonella* sp. was attempted from the gut according to standard methods for the detection of both motile and non motile Salmonellae. The intestinal contents were examined for parasites and processed by negative contrast electron microscopy for virus detection. Samples of livers were fixed in 10% buffered formalin for histology and histochemistry (Ziehl-Neelsen staining).

Results and discussion

In March 2008 a flock of 1,000 guinea fowls located in northern Italy experienced a sudden onset of a 0.8% daily mortality associated with depression. The guinea fowls, which were one hundred days hold, were housed in sheds provided with barriers against the entry of wild birds and in communication with an outdoor aviary. Pest control measures were regularly adopted and feed distribution took place inside the sheds. The flock was vaccinated for Newcastle disease and received a treatment for intestinal parasites.

At necropsy disseminated miliary necrosis of the liver and enlargement and marbling of the spleen were observed in five birds; three birds showed greenish diarrhoea.

Parasitological examination revealed the presence of intestinal Nematodes (*Ascaridia galli*) in three birds and flagellated protozoa in one bird.

Electron microscopy did not reveal the presence of any viruses.

Bacteriological examination of livers and spleens collected from five animals yielded a pure culture of non-haemolytic, Gram negative and oxidase negative bacteria identified as *Yersinia pseudotuberculosis*. The *Y. pseudotuberculosis* isolates showed resistances to colistin and spiramycin and sensitivities to the other tested molecules, including tetracycline, flumequine and apramycin.

Attempted isolation of *Salmonella* sp. gave negative result.

Histological examination of the liver and showed multifocal piogranulomatose lesions with purulent necrotic centre and infiltration of multinucleate giant cells: features ascribable to pseudotuberculosis.

The search of acid-alcohol resistant bacteria gave negative results.

One of the two birds kept under observation survived 2 weeks until spontaneous death. The second one was humanly sacrificed 6 weeks later. During the observation period they showed progressive wasting in spite of normal appetite. At necropsy cachexy, perihepatic cronic pedunculate lesions and hepatic hypoplasia were observed in both subjects.

On the basis of the results of the antimicrobial susceptibility test the birds received an in-feed tetracycline treatment.

The antibiotic therapy controlled effectively the disease and at the end of the withdrawal time the flock was slaughtered. Unfortunately we have no data concerning the condemnation rate at the abattoir, however a condemnation rate above the norm was reported in turkeys (Wallner-Pendleton and Cooper, 1982).

Conclusions

The pathological features observed in the carcasses of the guinea fowls submitted to our laboratory were consistent with a bacterial septicaemia and did not differ from those reported in other avian species affected by pseudotuberculosis (Harcourt-Brown, 1978; Wallner-Pendleton and Cooper, 1982). The bacteriological examination of livers and spleens yielded pure cultures of *Y. pseudotuberculosis*. The kind of farm with an outdoor aviary lead us to hypothesize that a contact with infected wild animals such as birds or mammal carriers, could have been the origin of the infection. The reported case suggests to consider Pseudotuberculosis among the diseases responsible for necrotic hepatitis in outdoor-reared guinea fowls.

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