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CASE REPORT

Food/farmed animals

Suspected *Laminosioptes cysticola* (fowl cyst mite) lesions in backyard chickens in southern England

Andy Grist[1] | **David G. Parsons**[2] | **Carlo Bianco**[3] | **Alessandra Cafiso**[4] | **Aiden P. Foster**[1]

[1]Bristol Veterinary School, University of Bristol, Langford House, Langford, UK

[2]The Poultry Health Centre, Trowbridge, UK

[3]School of Veterinary Medicine and Science, University of Nottingham Campus, Sutton Bonington, UK

[4]Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy

Correspondence
Aiden P. Foster, Bristol Veterinary School, University of Bristol, Langford House, Langford, North Somerset, BS40 5DU, UK.
Email: aiden.foster@bristol.ac.uk

Abstract

Postmortem examination of poultry from several backyard flocks in the south of England revealed five chickens (*Gallus gallus*) from one backyard flock with lesions compatible with the fowl cyst mite (*Laminosioptes cysticola*). All affected birds were mature hens and had subcutaneous and fascial, randomly scattered, 1–5 mm, calcareous nodules. Histological findings included a thin fibrous capsule, embedding glassy, pale basophilic acellular material that stained positive with von Kossa histochemistry consistent with mineralization. There were also some granulomatous lesions, detected in two of five birds, that were biconvex and made of an accumulation of amorphous eosinophilic material, collagen-like fibrillary material and fibroblasts, embedding some yellow to brown, refractile flakes and fragments of foreign bodies (suspected acarid exoskeletal remnants). Fresh and formalin-fixed tissues were examined by PCR for mite DNA with no positive results. *Laminosioptes* mites may be present in British backyard poultry.

BACKGROUND

As part of the veterinary degree programme at the University of Bristol, small-group postmortem examination of poultry is undertaken by students. Donated poultry carcases, from poultry processing plants and small backyard flock owners, are used to enable students to understand and perform postmortem examination techniques and become aware of common macroscopic lesions encountered. During these sessions, multiple, small, yellowish-white, calcareous, nodular subcutaneous lesions were observed, overlying the fascial planes of the muscles of the sternum, thigh and neck areas. Such lesions were considered typical of infestation with the fowl cyst mite (*Laminosioptes cysticola*), which was reported more than 40 years ago in the UK.[1]

The mite *L. cysticola* has worldwide distribution and has been reported in chickens, turkeys, goose, partridge, pigeon and wild Phasianidae. The life cycle is not well known: the adult mites live on the surface of muscles associated with small nodules under the skin; the nodules calcify after the mites die; and the females produce live larvae.[2] The mites may also be detected in the nervous tissue, abdominal viscera (kidney and liver), the peritoneum and lungs and air sacs.[2,3,8,19]

The aim of this case report is to describe the anatomical and pathological features of Laminosioptiosis in English chickens and to raise awareness that lesions consistent with *L. cysticola* mite infestation may be observed in British backyard poultry.

CASE PRESENTATION

Gross and histological examination

Five carcases were drawn from one backyard chicken flock located in one county in the south of England. They were examined for teaching purposes between 2015 and 2021 at the University of Bristol (see Table 1).

All the examined birds were 3–9 years old laying hens and had died from disease including egg peritonitis and traumatic injury. They displayed multifocal, subcutaneous and fascial, randomly scattered, small nodular lesions (diameter = 1–5 mm). They were presented as single nodules or small clusters; lesions were biconvex and flattened; the colour ranged from white to yellow; the consistency was hard (see Figures 1–4). There were no other significant macroscopic musculo-skeletal lesions or comorbidities in the chicken carcases; there were no nodular lesions similar to the subcutaneous and fascial ones on the serosal surfaces.

The nodular lesions and the surrounding cutaneous and fascial soft tissues were fixed in buffered formalin and processed for routine histology (staining with haematoxylin and

TABLE 1 Summary of chicken samples used in the study

Chicken number	Year sampled	Macroscopic lesions (mineralised nodules)	Histological findings (putative arthropod fragments present)	PCR
1	2015	Yes	Yes	Negative
2	2019	Yes	No	Negative
3	2021	Yes	Yes	Negative
4	2021	Yes	No	Not done
5	2021	Yes	No	Not done

FIGURE 1 Skin reflected from chicken (#3) with subcutaneous caseo-calcareous nodules visible on thigh and lateral thorax

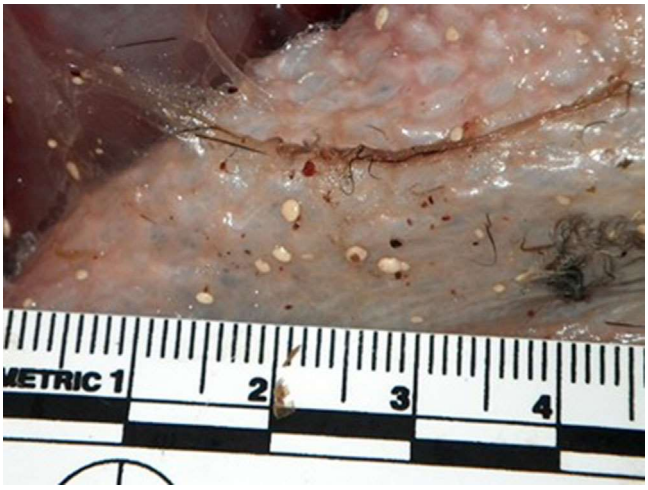

FIGURE 2 Nodules on skin and muscle, lateral aspect of thorax

eosin). To complement the histology, histochemistry was run on samples from chickens 2–5. The von Kossa histochemical stain was used, with and without prior decalcification, on samples from chicken 2, while von Kossa with no decalcification pre-treatment was performed on samples from chickens 3–5.

The histological findings were consistent across all the examined samples. There were two types of lesions observed: first, mineralised lesions detected in all of the birds examined, and second, smaller numbers of granulomatous lesions. The mineralised lesions were round to biconvex, attached to the fascial planes or expanding the dermis. They comprised a thin fibrous capsule, embedding glassy, pale basophilic acellular material (see Figure 5). There was a minimal inflammatory influx within the capsule, composed of plasma cells, histiocytes and lymphocytes. The basophilic material was positive (black) after von Kossa histochemistry, indicative of mineralisation. Ziehl–Neelsen staining of tissue sections, from three birds, was unrewarding with no acid fast bacilli identified.

The granulomatous lesions, compatible with the presence of mites and detected in birds 1 and 3, were attached to the fascial planes or located within the dermis; they were biconvex and composed of an accumulation of amorphous eosinophilic material, embedding some yellow to brown, refractile flakes and fragments of foreign bodies (suspected acarid exoskeletal remnants). The amorphous material was bounded by a thin fibrous layer, mildly infiltrated by some rare macrophages and other mononuclear cells including some lymphocytes, plasma cells and histiocytes (see Figure 6). In chicken 3, a small fascial lesion of degenerating leukocytes/cell debris embedding bacteria was also detected (see Table 1).

LEARNING POINTS/TAKE-HOME MESSAGES

1. *Laminosioptes cysticola* is a mite (also termed the fowl cyst mite) found in poultry and other birds and that resides below the skin; the life cycle is poorly understood; the mites do not cause significant clinical disease for the host bird.
2. Lesions associated with infestation are manifest as distinct subcuticular firm yellow-cream nodules that are usually associated with subcutaneous fascia overlying various muscles.
3. Mites are difficult to find and after death they become calcified nodules; remnants of such mites may be seen with histological sections. Mite identification based on PCR analysis of extracted DNA remains to be characterised.
4. Lesions are most likely to be seen in aged backyard laying hens and not originating from commercial flocks.
5. Previous reports on such mites in British poultry date back to 1977; veterinary surgeons should be aware that, at the time of writing, lesions associated with such mites may be detected on postmortem examination.

DNA studies

DNA was extracted from wax rolls (two wax rolls/sample; thickness = 20 μm), obtained from samples from chickens 1 and 3 with granulomatous lesions and presumed intralesional acarid fragments, using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions for formalin-fixed paraffin-embedded (FFPE) tissues. Fresh tissue (muscle and skin with lesional material from three birds) was subjected to DNA extraction using the DNeasy Blood and Tissue Kit following the manufacturer's instructions. Quantity and quality of the extracted DNA

samples was evaluated using a spectrophotometer (Nanodrop ND 1000; Thermo Scientific, Wilmington, DE, USA) and stored at −80°C until further analyses.

A control PCR was performed to evaluate the quality of the extracted DNA by amplifying a ~200 bp 12S rDNA gene fragment of the vertebrate host.[4] The presence of mite DNA was investigated using universal PCR primers for mites amplifying fragments of *Efla* (~400 bp) and 18S rDNA (~500 bp) genes[5] and universal arthropods PCR primers amplifying a ~157 bp fragment of the *COI* gene.[6] The PCR products were subjected to a reamplification step to increase the sensitivity of the methods.

The DNA of the vertebrate host was amplified in all FFPE and fresh tissues, suggesting a good DNA extraction and the absence of PCR inhibitors. The PCR analyses performed for mite DNA did not produce any results for both shorter (i.e. < 200 bp long) and longer PCR products (see Table 1).

DISCUSSION

Subcutaneous acariasis due to *L. cysticola* (Acariformes: Laminosioptidae, Vizioli, 1870) has had little attention paid to it in the UK and documented information is scarce. To the best of the authors' knowledge, the previous UK case report was in 1977;[1] here we have recorded cases of suspected Laminosioptiosis from a backyard flock in southern England over a period of 5 years.

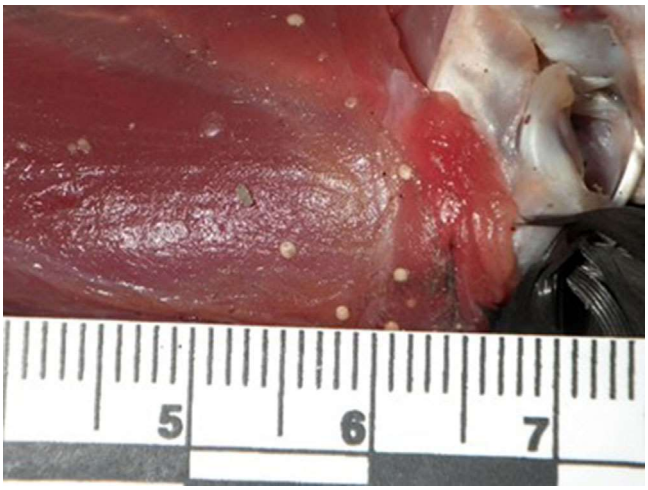

FIGURE 3 Nodules on outer aspect of thigh

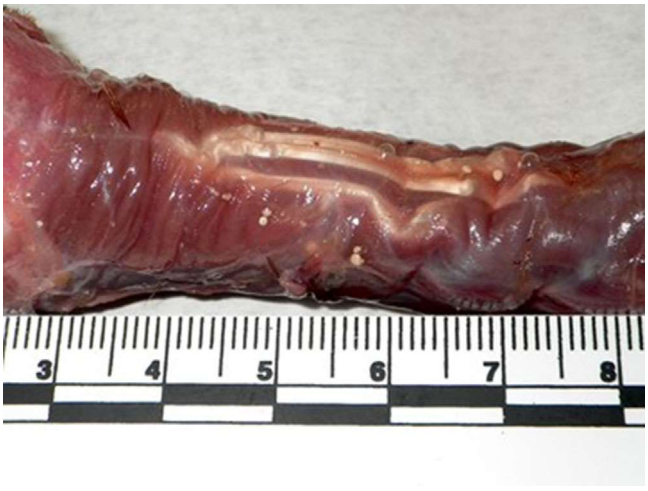

FIGURE 4 Nodules of *Laminosioptes* lesions on neck

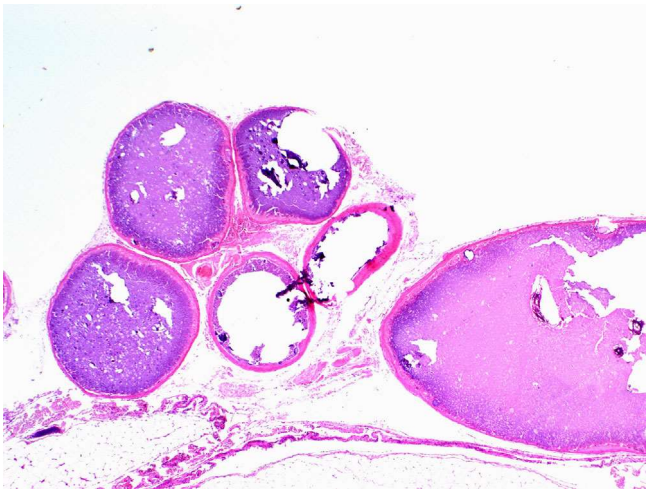

FIGURE 5 Chicken 1 typical histological appearance of subcutaneous nodules in chicken skin, which are made up of lakes of amorphous pale basophilic material encircled by a thin fibrous capsule embedding minimal inflammatory influx (haematoxylin and eosin; objective 2×)

There are few reports on the histological description of Laminosioptiosis in chickens and turkeys; our findings are similar to previous reports, withstanding the lack of mites.[7–10] The appearance of the mineralised lesions, detected in all the examined birds, was consistent with a chronic, quiescent and well-established process; the histological appearance of the granulomatous lesions suggested the presence of possible exoskeletal remnants within a chronic active process. In both cases, the intensity of the inflammation was minimal to mild, which is in keeping with the presumption that mite infestation is subclinical, with minimal pathological reaction. We processed multiple samples for histological examination, and the majority of the lesions were mineralised/calcified as previously reported. Previous studies have presumably detected mite infestation at an earlier stage of the life cycle, and it has enabled more convincing identification of the presence of mites.

We did not detect DNA for *Laminosioptes* mites and this may have been due to a number of factors. The mite bodies may have been too mineralized and degraded within the lesions to allow DNA amplification. It has been observed that DNA can be detected with a lower sensitivity in calcified cysts using PCR.[11] It may have been that mite DNA was degraded in the FFPE samples. Fragmentation and chemical modification of DNA due to formalin fixation and paraffin embedding has been shown to inhibit DNA amplification.[12] The PCR assays were performed using various universal primers, based on different specimens of both phylum Arthropoda and subclass Acari as positive controls. However, there are no known *Laminosioptes* spp. DNA sequences in GenBank database on which to design specific PCR primers, furthermore, there are no readily available *Laminosioptes* spp. positive control archived specimens for DNA extraction. Consequently, we cannot exclude the possibility that the amplification of *Laminosioptes* DNA in our samples was based on poorly specific PCR primers.

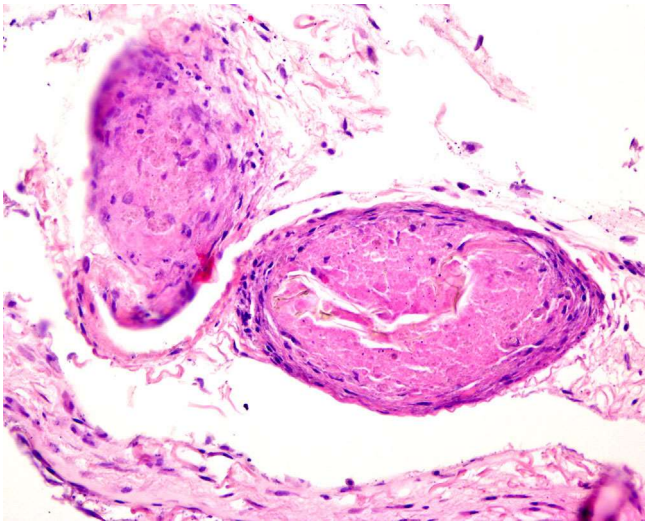

FIGURE 6 Chicken 1 histological appearance of a subcutaneous granuloma centred on necrotic materials embedding presumptive remnants of poorly refractile chitin, consistent with *Laminosioptes* mite infestation (haematoxylin and eosin; objective 20×)

Given previous reports, we contacted several authors in an attempt to access archived *Laminosioptes* mite material that could be used as a positive control for PCR analysis. Specimens were not available. Although there are techniques that can preserve arthropod DNA,[13] the advanced age and uncertain preservation of any museum specimens may make extraction of such DNA impossible; furthermore, processing such small specimens would likely have led to their destruction.

The mite seems to have a worldwide distribution and has been reported in chicken in Mexico,[10] India,[14] Brazil,[15] Zimbabwe,[16] Iran,[17,18] and Holland[19] and in chicken[20] and turkeys in the United States[9]; in the latter case, affected peripheral nerves produced torticollis in the live animal. It seems from the reports that the Laminosioptiosis does not cause significant clinical disease in the vast majority of cases and is detected incidentally.

The presence of numerous small nodules may render the carcase unfit for consumption and could lead to total rejection. It has to be acknowledged that normal routine meat inspection would not disclose the presence of such lesions. The postmortem inspection of poultry is performed on partially dressed, not skinned, carcases and is only visual. The so-called skin off or skinless poultry products are not inspected during meat inspection. This could in part explain why such lesions have not been reported in the past. Such lesions may be mistaken for subcutaneous fat deposits[15] or avian tuberculosis[3] or possibly xanthomatous lesions.[21] Consequently, histology is recommended to help establish the nature of the lesions.

A previous study[15] speculated on the possibility that consumption of free-range poultry meat containing the parasite may illicit a severe allergen reaction in hypersensitive humans due to the presence of the antigens tropomycin and chitin. It was suggested that allergy-induced anaphylaxis may occur with the consumption of acarid mite infested food and it was suggested that routine screening for sensitivity of Acaridae mite allergens should be considered in cases of human food allergy reactions.[22] The possibility of Acaridae allergen reaction in humans ingesting infected poultry would need further investigation, given that ingested mite allergens are usually associated with storage and house dust mite species.[23]

Further investigations would be needed to characterise the prevalence and the epidemiology of the suspected Laminosioptiosis in backyard flocks in the UK. More extensive histological sampling, including the major organ systems, may add further information about the clinical significance and the pathogenesis of Laminosioptiosis. The authors have not seen lesions in birds from commercial units including broilers, free-range layers or parent birds, possibly due to the younger age of those birds. With the number of flocks of less than 50 birds voluntarily registered in the UK reported as 19,197 and comprising 282,749 birds,[24] it may be the case that mature chickens in small free-range backyard flocks, with limited use of acaricides, are a potential source of this parasite (although wild birds might be a reservoir for these mites). There is limited information on the health status of backyard flocks in the UK.[25] Consequently, it was fortuitous that using the carcases for teaching purposes facilitated the detection of lesions in birds that otherwise would have been sent for disposal.

These findings would suggest that the fowl cyst mite should be considered in the postmortem examination of mature free-range poultry destined for human consumption.

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CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

FUNDING INFORMATION

This study was self-funded.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No formal ethical approval was required because the study was performed on material derived from postmortem examination used for teaching purposes.

AUTHOR CONTRIBUTIONS

Andy Grist and David G. Parsons provided the case material, history and gross images; they also initiated the study.

Carlo Bianco carried out the histological analyses. Alessandra Cafiso undertook the DNA studies. Aiden Foster arranged the histology and DNA studies, and drafted the manuscript. All of the authors reviewed and revised the manuscript.

ORCID

Andy Grist https://orcid.org/0000-0003-2496-8262
Carlo Bianco https://orcid.org/0000-0002-1693-1198
Alessandra Cafiso https://orcid.org/0000-0001-8739-3422
Aiden P. Foster https://orcid.org/0000-0003-3808-8434

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