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Spotty liver disease in Jordan: An emerging disease

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Abstract: Spotty liver disease is an acute bacterial disease that affects the poultry industry throughout the world. In this report, we discuss the first documented outbreak of the recently emerging disease, spotty liver disease, in a poultry flock in Jordan. The clinical history, pathological and molecular findings are described. The outbreak was characterised by recurrent mortalities that subsided with antibiotic treatments. Grossly, there were multiple pinpoint white foci distributed throughout the enlarged liver and less frequently throughout the spleen too. Histologically, the white foci represented areas of acute hepatocellular lytic necrosis and degeneration that were consistent with those of spotty liver disease. An end point polymerase chain reaction (PCR) assay targeting the glycerol kinase gene, coupled with sequencing, confirmed the pathological diagnosis. Continuous surveillance is needed to estimate the prevalence of this disease in Jordanian poultry flocks.

Keywords: *Campylobacter*; liver; poultry; spleen

Spotty liver disease (SLD) is an acute bacterial disease that is caused by *Campylobacter hepaticus* (Phung et al. 2020). SLD causes significant morbidity as well as mortality in poultry flocks throughout the world (Van et al. 2017b).

The disease was initially described in North America and was known as avian vibrionic hepatitis (Crawshaw 2019). The exact cause was believed to be of bacterial aetiology since no treatment was successfully effective other than the usage of antibiotics against this disease (Courtice et al. 2018). In 2015, a novel *Campylobacter* microorganism was isolated from different outbreaks of SLD and experimentally induced in specific-pathogen-free (SPF) chickens (Crawshaw et al. 2015). In 2016, a similar novel *Campylobacter* was isolated from outbreaks of SLD in Australia, but was fully characterised and was given a new name known as *C. hepaticus* (Van et al. 2016).

The disease is mostly reported as occurring in free-ranging flocks, however, it could occur in different housing systems (Scott et al. 2016). It could happen all year around with a 10% mortality rate among the flock (Grimes and Reece 2011). The disease is mostly characterised by its hepatic lesions that consist of a randomly distributed 1–2 mm white to grey foci of acute hepatocellular degeneration and necrosis (Crawshaw et al. 2015; Petrovska et al. 2017).

SLD had not been reported in poultry in Jordan. In this report, the pathological and molecular characterisation of a recently emerged outbreak of SLD in poultry in Jordan are discussed.

MATERIAL AND METHODS

Clinical findings and sample collection

The outbreak was recorded in a 40-week commercial free ranging brown layer farm. The flock consisted of 18 000 birds with 90% production. The disease started as an acute disease with increased

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bird mortalities and the egg production declined to 85%. Initially and before administration of any antibiotic, the daily mortality was 100 to 150 birds. With florfenicol administration, the daily mortality declined sharply to 30 birds and ended with zero mortalities after 5 days of treatment. Moreover, the egg production returned to its default production after the antibiotic treatment. The treatment ceased and the disease re-emerged after 3 weeks and a similar treatment scenario was repeated. Upon the post-mortem examinations, the dead birds exhibited hepatic lesions that consisted of miliary whitish foci distributed throughout the affected livers (Figure 1). Less frequently, the spleen was enlarged and mottled and had similar lesions as those seen in the affected livers (Figure 2). Less commonly, mild enteritis and enlarged kidneys were also seen. Different selected livers and spleens with lesions were sent to the microbiology and pathology laboratories for further evaluation and diagnosis.

Bacteriological examinations

The liver and spleen samples were subjected to routine aerobic and anaerobic bacteriological cultures. The aerobic culture was conducted on a blood and MacConkey agar while the anaerobic culture was conducted on a blood agar only.

Pathological examinations

The received tissue samples, namely the liver and spleen samples, were cut into thin sections and fixed in formalin for 24 hours. The fixed tissues were processed in an automatic tissue processor. The tissues were embedded in paraffin blocks, cut into 4 µm tissue sections and mounted on glass slides. The glass slides were stained with a haematoxylin and eosin stain (H&E) and evaluated by a certified veterinary pathologist and the results were recorded.

Molecular examination

The liver and spleen samples from the affected chicken were subjected to a genomic DNA extraction using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). After that, an end point polymerase chain reaction (PCR) using the primers (G2F3 and G2R2) was used to amplify a partial fragment of the glycerol kinase gene. The PCR and conditions were performed as mentioned previously (Van et al. 2017a).

The PCR products were separated in a 1.2% agarose gel. The PCR product band of 463 bp was excised and gel-purified then subjected to sequencing using a Big Dye v3.1 Terminator Kit (Applied Biosystems, Foster, CA, USA), using a SeqStudio genetic analyser (Thermo Fisher Scientific, Waltham, MA, USA). The sequences were edited using the BioEdit software v72. The sequence alignment was conducted between the partial glycerol kinase gene from a liver sample and a reference sequence (accession No. CP031611). The alignment was created using the T-Coffee tool (Di Tommaso et al. 2011).

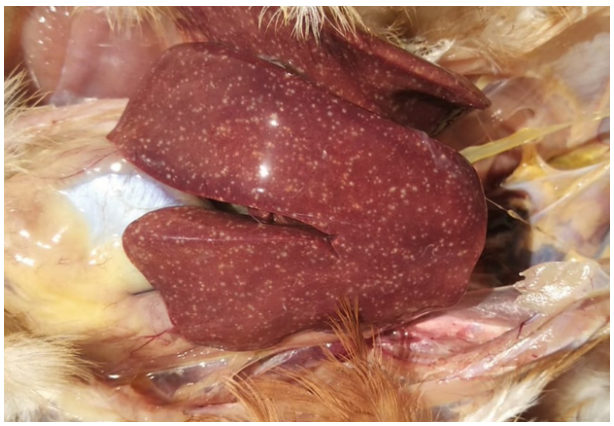

Figure 1. Avian, layer hen, liver. Widespread multifocal whitish foci are distributed throughout the liver

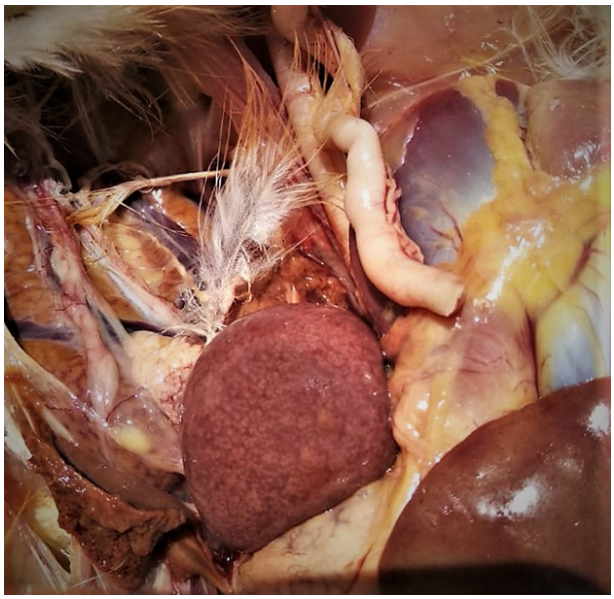

Figure 2. Avian, layer hen, spleen. Multifocal whitish foci are distributed throughout the enlarged spleen

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RESULTS

Bacteriological findings

The routine aerobic and anaerobic bacteriological cultures failed to isolate any organism from the submitted liver and spleen samples.

Pathological findings

Upon examination of the submitted livers and spleens, they exhibited pathological lesions as reported in the clinical history section. The histopathological examination revealed that the grossly visible whitish areas in the livers and spleens represented areas of necrosis. The liver sections showed randomly distributed areas of multifocal acute hepatocellular degeneration and liquefactive necrosis with a mild number of inflammatory cell infiltrates of primary heterophils (Figure 3).

In some areas, there were variably sized areas of fibrin deposition admixed with a few heterophils. The hepatic blood vessels were congested. The portal areas were mildly to moderately hypercellular with mononuclear cells. The Giemsa and Gram tissue stains failed to reveal any infectious process.

The spleen exhibited a disrupted splenic architecture caused by a moderate lymphoid depletion. The spleen exhibited multiple variably sized areas of necrosis and fibrin deposition throughout the examined sections (Figure 4).

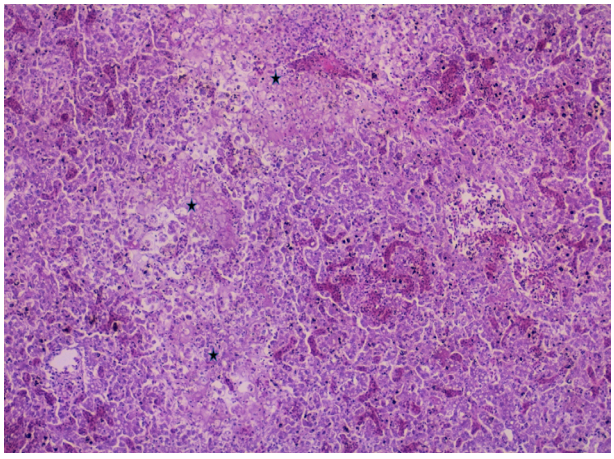

Figure 3. Avian, layer hen, liver. Multifocal to coalescing areas of acute hepatocellular necrosis with fibrin deposition and mild inflammatory cell infiltrates (★). H&E stain; magnification × 10

Molecular characterisation

Positive results were detected in the PCR and showed a positive band of 463 bp (Figure 5). Upon sequencing and alignment between the partial glycerol kinase gene from a liver sample and a reference

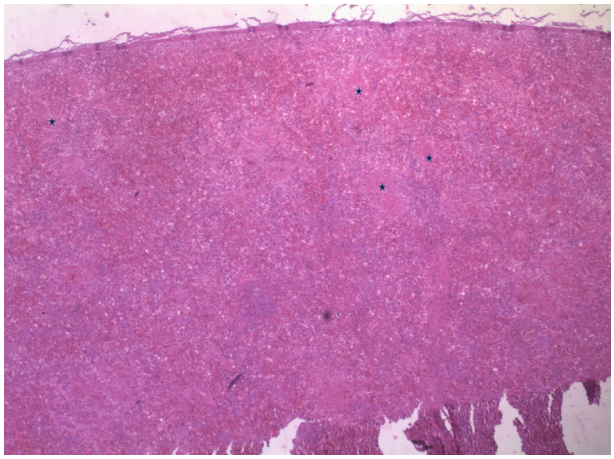

Figure 4. Avian, layer hen, liver. Throughout the spleen, there are multifocal areas of necrosis with fibrin deposition (★). H&E stain; magnification × 4

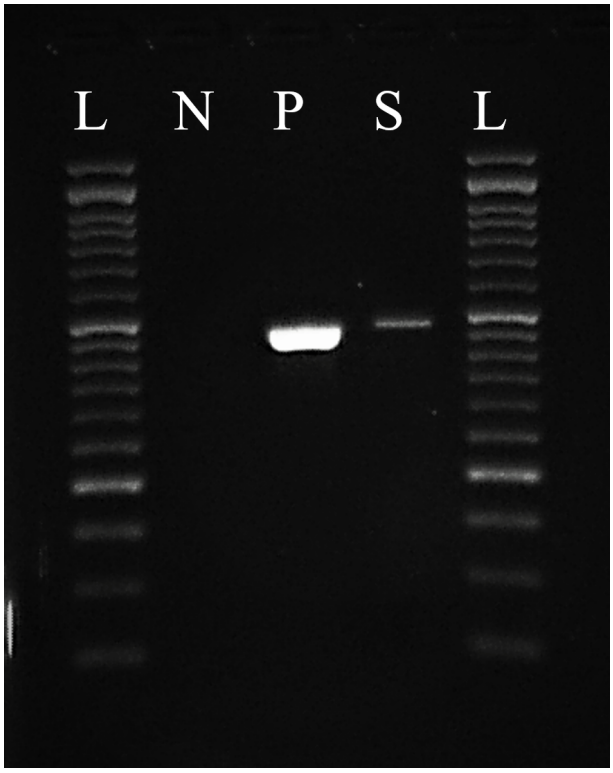

Figure 5. Gel electrophoresis image of the polymerase chain reaction product

L = DNA ladder which is 50 bp; N = negative control sample; P = positive control sample; S = examined liver sample from the diseased flock with a positive band of 463 bp

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Sample        61 TGCCTTTTTCAGTACACATTTGACCAAATAATGCAGCTTGTTGATCTCCGGCTATACCTG
CP031611.1    61 TGCCTTTTTCAGTACACATTTGACCAAATAATGCAGCTTGTTGATCTCCGGCTATACCTG

Sample       121 CTATTGGAATTTCACTATCAACCCATCTAGTAGCTGTATACCCATAAATTTCACTAGAAC
CP031611.1   121 CTATTGGAATTTCACTATCAACCCATCTAGTAGCTGTATACCCATAAATTTCACTAGAAC

Sample       181 TTTTAACTTCAGGAAGTATTGATCTTGGTATATCAAATAATTCAAGTAATTCATCATCCC
CP031611.1   181 TTTTAACTTCAGGAAGTATTGATCTTGGTATATCAAATAATTCAAGTAATTCATCATCCC

Sample       241 ATGATAAAGTATGGATATTATAAAGCATGGTTCTACTAGCATTGCTTACATCAGTTACAT
CP031611.1   241 ATGATAAAGTATGGATATTATAAAGCATGGTTCTACTAGCATTGCTTACATCAGTTACAT

Sample       301 GAATTTTCCCCTTAGTTAAATTAAAAATAAGCCAAGTATCTATAGTCCCAAAGCACAATT
CP031611.1   301 GAATTTTCCCCTTAGTTAAATTAAAAATAAGCCAAGTATCTATAGTCCCAAAGCACAATT

Sample       361 CTCCTTTTTTAGCTTTTAATCTTGCACCTTCTACATTATCTAATATCCA
CP031611.1   361 CTCCTTTTTTAGCTTTTAATCTTGCACCTTCTACATTATCTAATATCCA
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Figure 6. The sequence alignment between the partial glycerol kinase gene from a liver sample and a reference sequence of SLD (accession No. CP031611)

sequence (accession No. CP031611), the sequence matched the glycerol kinase partial gene of the reference *C. hepaticus* sequences in the GenBank (Figure 6).

DISCUSSION

This study was carried out to investigate the aetiology of a recent recurrent outbreak in a brown layer flock in Jordan. The clinical and pathological findings were consistent with previously reported outbreaks of SLD (Grimes and Reece 2011; Courtice et al. 2018). The molecular assays confirmed the clinical and pathological diagnosis of SLD.

SLD outbreaks occur most commonly in layer birds and usually occur in young birds (25-week-old) (Burch 2005; Crawshaw 2019). However, in one Australian study, it was reported that the mean flock age in differently occurring SLD outbreaks was 34-week-old (Courtice et al. 2018). In this outbreak, SLD occurred in 40-week-old brown layer birds. In this outbreak, no clinical signs of SLD were noted by the local veterinarian. The disease started as an increased mortality rate with no premonitory signs. Recently, it has been reported that birds can be infected with *C. hepaticus* without any clinical signs regardless of the onset of laying, without any clinical SLD (Phung et al. 2020).

The egg production of the farm was restored to its default production after treatment with florfenicol. Similar results were reported by another researcher (Courtice et al. 2018). However, other researchers reported that the egg production following the recovery from SLD would not be restored to its standard production as it was before getting SLD (Grimes and Reece 2011).

It had been reported that after an initial outbreak, the disease rarely re-emerges again (Grimes and Reece 2011). However, in this SLD outbreak, the disease recurred after the initial treatment with antibiotics. Similar findings of a recurring disease were also observed by Scott et al. (2016).

In the current study, the pathological lesions were similar to previously reported outbreaks (Crawshaw et al. 2015; Van et al. 2016; Courtice et al. 2018). Moreover, SLD was experimentally reproduced and induced hepatic and splenic lesions similar to these lesions reported in this study (Van et al. 2017b; Crawshaw 2019). In the experimentally infected birds, the pathological lesions were similar to those seen in field cases and were mainly comprised of multifocal necrotising hepatitis (Van et al. 2017b; Crawshaw 2019).

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The exact source of the SLD in this farm could not be determined, however, it was reported that biosecurity measures and different vectors could play a role in the transmission of *C. hepaticus* to the birds (Scott et al. 2018; Phung et al. 2020). Phung et al. (2020) isolated the *C. hepaticus* DNA from different environments including the faeces from wild birds and rats in addition to flea samples of SLD positive farms. The authors proposed that organisms act like a vector in the transmission of *C. hepaticus* to the birds. In another study, it was found that wild birds that were commonly present in the birds' farms act as a potential vector for the transmission of *C. hepaticus* to the birds and the subsequent SLD (Scott et al. 2018).

To date, to the best of the authors' knowledge, this is the first reported outbreak of SLD in the poultry industry in Jordan. Veterinarians should be aware of this newly emerging infectious disease in layer farms in Jordan. The best farm management and a strict biosecurity practice and antibiotics help in the control of the disease.

Conflict of interest

The authors declare no conflict of interest.

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