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Outbreak of encephalitic listeriosis in red-legged partridges (*Alectoris rufa*)

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Abstract

An outbreak of neurological disease was investigated in red-legged partridges between 8 and 28 days of age. Clinical signs included torticollis, head tilt and incoordination and over an initial 8 day period approximately 30-40 fatalities occurred per day. No significant gross post mortem findings were detected. Histopathological examination of the brain and bacterial cultures followed by partial sequencing confirmed a diagnosis of encephalitis due to *Listeria monocytogenes*. Further isolates were obtained from follow-up carcasses, environmental samples and pooled tissue samples of newly imported day-old chicks prior to placement on farm. These isolates had the same antibiotic resistance pattern as the isolate of the initial post mortem submission and belonged to the same fluorescent amplified fragment length polymorphism (fAFLP) subtype. This suggested that the isolates were very closely related or identical and that the pathogen had entered the farm with the imported day-old chicks, resulting in disease manifestation in partridges between 8 and 28 days of age. Reports of outbreaks of encephalitic listeriosis in avian species are rare and this is to the best of our knowledge the first reported outbreak in red-legged partridges.

Key words: *Listeria monocytogenes*, listeriosis, partridge, encephalitis, outbreak, avian listeriosis, game birds, encephalitic listeriosis

Introduction

The genus *Listeria* consists of 10 species, two of which have been associated with disease in humans and animals (Orsi et al., 2011; Bertsch et al., 2013; Lang Halter et al., 2013). Disease due to *Listeria ivanovii* appears to be rare and restricted to

ruminants (Orsi et al., 2011; Low & Donachie, 1997). In contrast *Listeria monocytogenes* is associated with disease in a variety of animal species and man (Gray, 1958; Low & Donachie, 1997). *L. monocytogenes* has a worldwide distribution and is widespread within the environment (Gray & Killinger, 1966). There are 16 known serotypes but only three have been commonly described in context with disease which include 1/2a, 1/2b and 4b (Low & Donachie, 1997; Seeliger & Jones, 1986). Phylogenetically *Listeria* spp are divided into four different lineages with 1/2b and 4b predominantly belonging to lineage I, and 1/2a belonging to lineage II (Orsi et al., 2011).

Interest in *L. monocytogenes* increased in the 1980s when it was recognised as a significant food-borne pathogen causing disease in humans (Low & Donachie, 1997).

In animals, clinically significant infections with *L. monocytogenes* are mostly recognised in ruminants (Low & Donachie, 1997). They have also been described in over 17 avian species including chickens, turkeys, geese, ducks, canaries, parrots and others (Gray & Killinger, 1966). However, listeriosis in birds appears to be rare and outbreaks are sporadic with varying morbidity and mortality (Gray, 1958). Birds are considered difficult to infect with *L. monocytogenes* and young birds are considered most susceptible to disease (Bolin, 1960; Basher et al., 1984). Listeriosis in birds mostly presents as septicaemia with splenomegaly, necrosis of liver and myocardium as well as pericarditis (Gray, 1958; Barnes & Nolan, 2008). Reports of outbreaks of avian encephalitic listeriosis are rare and originate mostly from chickens in the USA, Japan and India (Cooper, 1989; Cooper et al., 1992; Kurazono et al., 2003; Vijayakrishna et al., 2000). Wet weather (flooding), de-beaking or injections into the neck were reported to be likely predisposing factors. Torticollis, depression and incoordination

were a consistent clinical sign in affected birds. These neurological signs cannot be distinguished from those of the notifiable Newcastle Disease, which should be considered a potential differential diagnosis to encephalitic listeriosis (Irvine et al., 2009).

In the UK there have been occasional publications on the isolation of *L. monocytogenes* from avian species including chickens (*Gallus gallus domesticus*) (Paterson, 1937; Paterson, 1939), wild grey partridges (*Perdix perdix*) in the vicinity of an infected sheep flock (McDiarmid, 1961) and a merlin (*Falco columbarius*) that showed neurological signs after having eaten a sparrow presenting with neurological signs (Baker, 1967).

The only report found on listeriosis in red-legged partridges refers to the isolation of *L. monocytogenes* from two partridges in France in 1957 without providing any further information (Lucas & Seeliger, 1957).

This report describes the first case of encephalitic listeriosis affecting young red-legged partridges.

Materials and Methods

Background: In May 2011 four 16-day-old red-legged partridges (*Alectoris rufa*) with neurological clinical signs were submitted to the Royal Veterinary College – Animal Health and Veterinary Laboratory Agency (RVC-AHVLA) Surveillance Centre London for post mortem examination. The partridges originated from a game bird rearing farm that imported red-legged partridges as day-old chicks every two weeks during the season. A total of four batches (A-D) varying in size of between 15,000 and 30,000 were imported in 2011. The diseased 16-day-old partridge chicks were from batch A's

25,000 birds which had lost approximately 30-40 chicks daily over the last eight days. Some birds were found dead but the majority exhibited neurological signs resulting in an inability to use the nipple drinkers. They subsequently either died or were culled. Three sheds out of a total of 20 appeared to be particularly affected, whereas only occasional affected birds were detected in the remaining sheds. There was no improvement following treatment with various antibiotics. However losses started dropping from approximately four weeks of age onwards. Total losses in the first and worst affected batch A reached 5.2% at eight weeks of age (in previous years total losses in partridges on this farm had been approximately 2.5%- 3% at 13 weeks). Three subsequent batches of red-legged partridges were also affected in the same age range but with a much lower morbidity. Their total mortality rate was within expectations for age and production type.

Further carcasses of affected birds from the original batch A and the second affected batch B were also submitted for post mortem examination at a later date as were day-old partridges of batch D that had died during transit and had never entered the sheds on the farm. A summary of submitted birds can be seen in table 1.

Sheep had been grazing the ground over the winter. However, partridges had no access to outside runs until four-week of age and were kept indoors on cardboard squares on top of a continuous polythene sheet. There was a good level of biosecurity and cleanliness on the farm. Drinking water was supplied from the mains via holding tanks together with electrolytes and multivitamin solution. The weather had been unusually dry and warm during the outbreak and there had been no flooding. Data from the trade control and expert system (TRACES) (a management system of the veterinary authorities to track animal imports within the EU and from outside) showed

that the investigated farm was the only farm in the UK that had received day-old partridge chicks from a specific supplier when the first and worst affected batch (A) arrived. Other farms in the UK received day-old partridges from the same supplier only more than a week later.

Gross pathology and histopathology: Routine post mortem examinations were performed on the submitted partridges as detailed in table 1. Live partridges were euthanased by cervical disarticulation following clinical examination. Swabs of brain and/ or liver were taken from a total of nine birds and stored in charcoal transport medium until microbiological culturing. Small tissue samples of liver, lung, yolk sac and brain were removed from six one-day-old partridges of batch D that had died during import transit and were pooled in buffered peptone water prior to microbiological culture. Various tissue samples including brains were collected in 10% formol saline and processed for routine histopathology.

Sections were prepared, embedded in paraffin wax, serially cut(5µm) and mounted on glass microscope slides. They were stained with haematoxylin and eosin, special Gram stains were also undertaken for some of the sections.

Environmental sampling: Bacteriological swabs were taken from the lining of the delivery boxes of the day-old imported partridge chicks of batch C during the follow up submission. Subsequently, a farm visit was carried out to collect epidemiological data and environmental samples. Sterile gauze swabs were moistened with buffered peptone water and swabs were taken from the floor of sheds affected by disease of two different batches (A and C), a shed with birds of batch A that had not been

affected by disease, straw of an affected shed and clean straw from the storage stack. Aliquots were taken from drinking water, multivitamin solution and two types of feeding pellets. A summary of all collected environmental samples can be seen in table 2.

Microbiology: Routine cultures of samples took place on Columbia Blood agar (BA, Oxoid, Basingstoke, UK) and MacConkey agar (MAC, Oxoid) at 37°C. Bacteria were identified using standard laboratory techniques. *L. monocytogenes* was identified by colonial morphology as beta-haemolytic colonies on blood agar and small lactose fermenting colonies on MacConkey agar. They were Gram-positive short rods and catalase test positive. The colonies were confirmed as *L. monocytogenes* either by API Listeria (bioMérieux, Marcy-l'Etoile, France) or the CAMP test. (*L. monocytogenes* is CAMP test positive, *L. innocua* is CAMP test negative (McKellar, 1994)).

Many samples were additionally or only cultured in selective media: they were cultured directly onto *Listeria* Selective Agar (LSA, Oxoid) using a swab. Additionally 1g or 1ml of samples (depending on sample type) was inoculated into *Listeria* Selective Enrichment Broth (LSB, Oxoid). The LSA plates were incubated overnight at 30°C and daily examined for colonies typical of *Listeria*. The LSB was incubated at 30°C and subcultured after 24 hours onto fresh LSA and again after 48 hours. The subculture plates were examined after 24 and 48 hours for *Listeria*-like colonies as described above. Where *Listeria*-like colonies were isolated on LSA they were subcultured on to BA and MAC for further confirmation as described above.

Box liners and environmental samples were pre-enriched in buffered peptone water at 37°C overnight and then cultured directly onto LSA and transferred into LSB.

Antibiotic Sensitivity: Two isolates of *L. monocytogenes* isolates were tested against a panel of eight antibiotics, namely Ampicillin (10µg), Doxycycline (30µg), Enrofloxacin (5µg), Lincomycin (10µg), Penicillin (1µg), Tetracycline (10µg), Tylosin (30µg) and Trimethoprim/ Sulphamethoxazole (25µg), supplied as 6mm impregnated discs by Oxoid. They were placed on an Oxoid lysed blood sensitivity agar plate together with emulsified colonies and the plate was incubated for 18-20 hours at 37°C. The zones of inhibition around each antibiotic disc were measured using an automated ProtoZone sensitivity reader (Don Whitley Scientific, Shipley, UK). Resistance was denoted by a total zone diameter of 13mm or less. Resistance gene testing was performed by using an antimicrobial resistance gene microarray for Gram positive organisms that is available through Alere Technologies (Jena, Germany). Genomic DNA was isolated as described by Perreten et al. (2005). Approximately 100 ng of DNA was labelled by a randomly primed polymerization reaction using Sequenase, version 2.0 (USB Corporation, Cleveland, Ohio) and consisted of three cycles of enzymatic reactions (Bohlander et al., 1992). This was followed by hybridisation of the labelled fragments to the array following the protocol of Perreten et al (2005). The arrays were subsequently scanned and the IconoClust software (Alere^{TM}) used to identify positive signals.

Partial sequencing: In order to provide preliminary molecular confirmation of isolate identity the virtually complete 16S rRNA gene was amplified from the initial *L. monocytogenes* isolate of the brain of a red-legged partridge (bird 1Ab) by PCR using primer pair 5’ AGTTTGATCCTGGCTCAG 3’ and 5’ ACCTTGTTACGACTT 3’ and sequenced

using a series of internal primers as described previously (Hunt et al., 2013). Phylogenetic analysis previously described by Graves et al. (2010) was reproduced to confirm placement of the isolate relative to reference strains of *Listeria*. Phylogenetic analysis was performed using the MEGA5 program (Tamura et al., 2011) as described in figure legends. In order to further characterise phylogenetic position, sequencing of fragments of additional housekeeping genes (*sigB*, *gap* and *prs*) was also performed as described by Graves et al. (2010) and phylogenetic analysis was performed as above with reference to the sequences included in Graves et al. (2010).

Serogrouping and fAFLP: On receipt at the National reference Laboratory (NRL) for *Listeria* (Public Health England, London, UK), *Listeria* isolates were cultured on blood nutrient agar (BN) and incubated at 37°C for 12-24h. Bacterial nucleic acid was extracted from an isolated colony grown on BN plate, using MicroLYSIS™ reagent (Microzone Ltd, Haywards Heath, UK). Briefly, a 1µl loop full of bacterial growth was mixed with 19µl of MicroLYSIS solution. The mixture was then subjected to 65°C for 5 minutes, 96°C for 2 minutes, 65°C for 4 minutes, 96°C for 1 minute, 65°C for 1 minute and 96°C for 30 seconds and used directly for PCR.

L. monocytogenes was identified using an in-house duplex real time PCR exonuclease-assay (LM-PCR) amplifying simultaneously a specific 112 bp fragment of the *L. monocytogenes* haemolysin gene (*hly*A) and a specific 80bp fragment of the *L. monocytogenes* phospholipase A (*plc*A) gene (Nogva et al., 2000). Each reaction contained 25µl of 1x TaqMan™ Fast Universal PCR Master Mix (Applied Biosystems, Life Technologies, California, US), 0.3µM of each of the forward and reverse primers (Eurogentec, Seraing Belgium), 0.1µM of each fluorescently labelled probes

(Eurogentec) and 5µl of crude or purified DNA extract. The reaction was performed under 'FAST' default conditions on an Applied Biosystems 7500 FAST Sequence Detection System ('TaqMan') according to the manufacturer's protocol.

Molecular serogrouping of *L. monocytogenes* isolates was performed using the multiplex gel-based PCR assay (LSER-PCR) described by Doumith et al. (2004) using 5µl of DNA extract. Pre-cast ethidium bromide-stained 1.5% agarose E-gel© (Invitrogen, Life Technologies) were used to separate the multiplex PCR products by electrophoresis. Results of the electrophoresis were visualised under UV light and photographed using a BioRad™ UV Gel-doc© system (Hemel Hempstead, UK). According to the pattern obtained (Doumith et al., 2004; Huang et al., 2011) isolates were classified as being one of the four *L. monocytogenes* serogroups, as *Listeria* spp. or as being non *Listeria* species.

Fluorescent amplified fragment length polymorphism (fAFLP) technique was used to sub-type *L. monocytogenes* isolates and was performed using a modification of a protocol previously described for *Campylobacter* (Desai et al., 2001). Briefly, genomic DNA (15-50ng) was digested with 5U of each of two restriction enzymes, *Hind*III and *Hha*I, (New England Biolabs, Hitchin UK) in the presence of RNase A and bovine serum albumin. Digested fragments were ligated to two sets of specifically designed double stranded adapters. These adapters served as targets for a FAM-labelled Hind-A and a non-labelled Hha-A selective primers (Eurogentec, Seraing Belgium) for fragment amplification by PCR. PCR products were separated on an ABi 3730XL 96capillary DNA Analyzer (Applied Biosystems) alongside a GeneScan™- 600 LIZ™ Size standard, and chromatographs showing FAM-fluorescing fragments were saved as .fsa files. The .fsa files were exported, visualised and analysed on PEAK SCANNER™ v1.0 (Applied

Biosystems, Life Technologies, Paisley UK). As well as the chromatographs, PEAK SCANNER™ also recorded the fragment data in a binary format in Excel files. These files were then exported into Bionumerics v6.1 where they were visualised as virtual electrophoresis gels and analysed. The patterns determining the fAFLP types were identified using in-house built libraries.

Results

Clinical signs: All four live affected red-legged partridge chicks in the first submission exhibited marked neurological signs including torticollis, head tilt and incoordination (Fig. 1).

Gross post mortem findings: Other than septicaemia with perihepatitis and pericarditis in one dead carcass (bird 2Be) and pneumonia concurrent with neurological signs in one live bird (bird 1Aa), there were no significant gross post mortem findings in the remaining carcasses. The majority of the birds had empty crops and gizzards and slightly prominent ureters. Given the clinical history and the absence of clear gross post mortem findings, the case was notified to Animal Health as suspect Newcastle Disease as required under EU council directive 92/66/EEC. Newcastle disease was ruled out following official laboratory tests carried out by government laboratories.

Histopathological findings: Examination of brain tissues revealed focal sub-acute encephalitis of the brainstem and cerebellum with gliosis and perivascular cuffing. In addition, a microabscess was detected in one of those brains. In two of the brains Gram positive bacilli were associated with the lesions (Fig. 2). Other examined tissues

including heart, lung, liver, intestine and sections of spinal cord were mostly unremarkable other than concurrent focal fibrinogranulocytic pneumonia and bronchitis with bacterial infection and possibly inhaled foreign plant material in one of the birds (1Aa).

Microbiological findings: The bacteriological results are illustrated in table 1 and 2. In summary, *L. monocytogenes* was isolated on routine bacteriological culture in mixed growth from one of two brain swabs in submission 1 and on direct culture in selective media from three out of four brain swabs in submission 2. This also included a swab from the brain of a partridge belonging to the second (younger) Batch B. Following enrichment and selective culture *L. monocytogenes* was also isolated from multiple environmental samples including from the sheds of different batches (A and C) of partridges regardless of whether there was a high level of neurological disease in the shed or not. However, no *Listeria* spp were isolated from samples of the drinking water, feed or multivitamin solution. *L. innocua* was isolated from the clean straw from the storage area that served as source for the straw used in the corners of the sheds. No *Listeria* spp were isolated from the transit boxes. However, *L. monocytogenes* was isolated from an organ pool (liver, lung, yolk sac and brain) of six one-day-old partridges of the fourth batch (D) that had died during import transit and never entered the farm sheds.

Antibiotic resistance: The original *L. moncytogenes* isolate from the brain of partridge b of the first submission and the *L. monocytogenes* isolate from the organ pool of the day-old partridges both underwent testing for antibiotic sensitivity, showing

resistance to Doxycycline and Tetracycline and sensitivity to Trimethoprim/ Sulphamethoxazole, Lincomycin, Tylosin, Ampicillin, Penicillin and Enrofloxacin.

Gene array analysis revealed that both isolates were positive for both probes for the *tet*M gene (both also single positive for *tet*Z; the 1Ab isolate had a single positive probe for *mup*R and *van*D; the day-old isolate had a single positive probe for *tet*L).

Partial sequencing results: Molecular sequence analysis was carried out on the original isolate from partridge 1Ab to confirm identity of the strain and to establish its relationship with other *Listeria* isolates. Analysis was carried out in comparison with a recent detailed analysis that described a novel species *Listeria marthii* (Graves et al., 2010). Sequencing and phylogenetic analysis of the partial 16S rRNA gene, as shown in Figure 3, demonstrated that the isolate is most closely related to *L. monocytogenes* strains including the type strain NCTC 10357. In order to obtain more phylogenetic information a more comprehensive analysis was carried out using concatenated sequences of three housekeeping genes (*sigB*, *prs* and *gap*) and undertaking direct comparison with previous analyses (Graves et al., 2010). As illustrated in Figure 4 these analyses provided more precise description of the isolate placing it clearly within lineage II of *L. monocytogenes*.

Fluorescent Amplified Fragment Length Polymorphism (fAFLP) results: Five isolates of *L. monocytogenes* from this outbreak in partridges including samples from brain swabs and environment underwent serogrouping and fAFLP testing at the NRL.

Additionally, a few *L. monocytogenes* isolates of animal origin kept in the AHVLA bacterial strain collection underwent similar analysis. These additional isolates included one from a partridge heart collected in 1992, two from pheasant livers of 1998 and 2002, two from chicken organs, as well as isolates from the brain of cattle, sheep and goat. The results as illustrated in Fig. 5 revealed that the majority of animal samples including all partridge samples belonged to serogroup 1/2a with fewer belonging to 1/2b or 4. Interestingly, all partridge isolates from the current outbreak were in the same fAFLP group VIIa.84. This group has not previously been detected in the UK. The other isolate from a partridge in 1992 was related but not identical with a similarity of 83.8% (fAFLP group: VIIa.85). In contrast all other samples of animal or human origin were not closely related.

Discussion

To the best of our knowledge this is the first report of an outbreak of encephalitic avian listeriosis in Great Britain and the first one in red-legged partridges worldwide. Previous outbreaks of avian encephalitic listeriosis have so far been reported in chickens and had morbidities <0.5% (Cooper, 1989; Cooper et al., 1992; Kurazono et al., 2003). The estimated morbidity of encephalitic listeriosis in the first batch of red-legged partridges in this case was higher and approximated 3%. In the subsequent three batches however, estimated morbidity of neurological disease dropped continuously and could not be clearly separated from the expected background mortality for partridges of this age.

Clinical, microbiological and histopathological findings as well as the absence of gross post mortem findings were consistent with encephalitic listeriosis. In line with

334 previous publications, isolation of *L. monocytogenes* was restricted to swabs from the
335 brain and there was mixed growth on routine cultures in the initial submission, from
336 which *L. monocytogenes* was isolated (Gray, 1958; Cooper, 1989). The success rate for
337 isolating *L. monocytogenes* improved when media selective for *Listeria* spp and
338 enrichment steps were used and swabs were taken from the brainstem area of the
339 birds.

340 The original isolate was confirmed as *L. monocytogenes* by 16S RNA sequencing.
341 Additional sequencing placed it into Lineage II, one of at least four evolutionary
342 lineages of *L. monocytogenes* that is common in foods, widespread in natural and farm
343 isolates and commonly associated with animal listeriosis cases as well as sporadic
344 human cases (Orsi et al., 2011). In contrast most human listeriosis outbreaks are
345 associated with lineage I isolates. Lineage II isolates are most commonly associated
346 with serotypes 1/2a, 1/2c and 3a and are notable for extensive plasmid carriage and
347 associated resistance to environmental compounds as well as bacteriocin resistance
348 (Orsi et al, 2011). In this context, it was interesting to note that the *L. monocytogenes*
349 isolate from the partridge was resistant to tetracyclines in spite of antibiotic resistance
350 to tetracyclines being considered uncommon amongst *Listeria* spp. High levels of
351 tetracyclines are even recommended for treatment of the disease by some authors
352 (Barnes & Nolan, 2008). Morvan et al. (2010) showed that 0.77% of *Listeria*
353 *monocytogenes* isolates from humans in France were resistant to tetracyclines.
354 The main questions that remained following the initial diagnosis in this outbreak
355 included: how did the pathogen enter the farm and the partridges; how did it spread;
356 why did it cause disease in so many birds; and why was disease restricted to the
357 nervous system in spite of most earlier publications referring to listeriosis causing
358 septicaemic disease in avian species (Gray, 1958).

359 *L. monocytogenes* has been shown to be widespread in the environment including in
360 the soil and silage and many species including sheep, cattle and avian species have

been shown to harbour it in and excrete it from the intestine without any clinical signs (Low& Donachie 1997). Oral infection with the pathogen is considered most common (Gray & Killinger, 1966). It was therefore surprising that the diseased partridges had not had any soil contact prior to disease development. There was also no indication of the involvement of water or feed. However, there was widespread presence of the pathogen in the indoor environment of the sheds, identifying this as potential mode of spread for disease from infected birds. No *L. monocytogenes* was isolated from the clean straw prior to being used in the sheds and this was therefore unlikely to have introduced the pathogen into the sheds.

Interestingly, *L. monocytogenes* was isolated from an organ pool of day-old imported partridges that had never entered the farm buildings suggesting the possibility that the pathogen was introduced into the farm by imported newly hatched birds that might have been infected in the hatchery or during transit. This theory would also potentially provide an explanation for the differences in morbidity in the various sheds depending on where the infected birds had been placed. Wet weather, flooding, de-beaking and injections have been considered predisposing factors for disease in previous avian outbreaks of encephalitic listeriosis with flooding having been suspected as the potential mode of pathogen distribution (Cooper et al., 1992; Kurazono et al., 2003). However, neither weather conditions nor management factors are believed to have played a significant role in this case given a very dry and warm spring and good management and biosecurity levels on the farm. Horizontal spread of infection from in-contacts is therefore being hypothesised. Birds are considered difficult to infect with *L. monocytogenes,* however, Basher et al. (1984) found that

384 newly hatched chicks could be infected by natural contact to experimentally infected
385 young chicks and subsequently develop disease and die.
386 Additional testing supported the theory that the pathogen had been introduced into
387 the farm by imported day-old chicks by identifying a similar antibiotic resistance
388 pattern between the different *L. monocytogenes* isolates with resistance to
389 tetracyclines likely due to the *tet*(M) gene..
390 The bacterial isolates from this outbreak also all belonged to serogroup 1/2a, which is
391 commonly associated with clinical cases of listeriosis in animals in contrast to
392 outbreaks in humans that are mostly associated with serogroups 1/2b or 4b from
393 lineage I (Orsi et al., 2011). Interestingly, the isolates from the previously reported
394 outbreaks of avian encephalitic listeriosis in chickens in the USA and Japan all
395 belonged to serogroup 4b (Cooper, 1989; Cooper et al., 1992; Kurazono et al., 2003),
396 as did one of the isolates from red-legged partridges in France in 1957; the other
397 isolate was no further classified than serogroup 1 (Lucas & Seeliger 1957). These had
398 been to the best of our knowledge the only reported *L. monocytogenes* isolates from
399 red-legged partridges worldwide.
400 Further subtyping by fAFLP revealed that all isolates from this outbreak also belonged
401 to the same fAFLP type: VIIa.84. This fAFLP type was different to any previously
402 identified by Public Health England (Corinne Amar, personal communication). It was
403 also different to other animal isolates analysed at the same time. Most closely related
404 was an isolate collected from the heart of a partridge from a different farm in 1992
405 and analysed now at the same time that had 83.8% similarity in its fAFLP profile. This
406 raises the question as to whether a partridge specific strain of *L. monocytogenes* might
407 exist. Unfortunately, this was the only *L. monocytogenes* isolate of a partridge

408 available from the AHVLA bead collection due to the rarity of diagnosed listeriosis in
409 avian species in the UK. Isolates from pheasants, chickens and brains of ruminants
410 could be classified into different fAFLP groups and some even different serogroups
411 e.g. 1/2b or 4.
412 This very interesting and unusual case left several questions unanswered. It remains
413 unclear what underlying factors allowed disease to develop to such an extent and why
414 it manifested itself as neurological disease. There was no indication for visceral disease
415 caused by *L. monocytogenes*. It also remained unclear why there had been no similar
416 reports from other farms though the absence of additional imports from the same
417 supplier source at the same time as the first batch was acquired might account for
418 this. The morbidity in subsequent batches was much lower and cases might have been
419 missed amongst "normal" mortality rates.
420
421 *L. monocytogenes* is a well-recognised zoonotic pathogen but the zoonotic risk in this
422 case was considered low as the majority (99%) of human infections are foodborne
423 (Mead et al., 1999; Swaminathan & Gerner-Smidt, 2007). There have however been
424 reports of cutaneous or ocular infection due to contact with infected animals
425 (McLauchlin et al., 2004) and the farmer and his workers were made aware of
426 potential risks. No human health issues were observed during this outbreak.
427 A further important aspect of this outbreak with marked neurological signs and a rise
428 in mortality was that avian notifiable disease, specifically Newcastle Disease, could not
429 be ruled out on clinical grounds, notification of suspect disease took place and
430 subsequent official laboratory tests were required. There can be a large variation in
431 the nature and severity of clinical signs seen in cases of avian notifiable disease

(Newcastle disease or Avian Influenza). Therefore any progressive neurological signs in poultry and game birds coupled with an unexplained rise in mortality or drop in production should prompt the consideration of avian notifiable disease as an alternative differential diagnosis (Irvine et al., 2009).

On the basis of the findings in this report, listeriosis should also be considered an unusual but potential cause for neurological signs in avian species – not only in individual birds but also in an outbreak-like situation.

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Table legends:

Table 1: Summary of bacteriological findings, brain histopathology and other findings in the submitted red-legged partridges. Live submitted birds were showing neurological signs.

Submission (date)	Batch	Individual identifier	Age (days)	Bacteriology	Histopathology of brain	Other
1 (06/05/11)	A	Bird 1Aa (live)	16	ND	Focal subacute encephalitis of the brainstem and cerebellum with associated Gram positive bacilli	Acute pneumonia and bronchitis
1 (06/05/11)	A	Bird 1Ab (live)	16	Brain: mixed growth including Lm on routine culture	As above	No growth on fungal culture; liver lead: 3.2µmol/kg DM
1 (06/05/11)	A	Bird 1Ac (live)	16	Brain: NSO Liver: NSO	As above but no Gram positive bacilli visible	No growth on fungal culture
1 (06/05/11)	A	Bird 1Ad (live)	16	Liver: NG	As above with no Gram positive bacilli visible but microabscess in one section	
2 (18/05/11)	A	Bird 2Aa (live)	28	Brain: mixed incl Lm on LSA	ND	
2 (18/05/11)	A	Bird 2Ab (dead)	28	Brain: mixed incl Lm on LSA	ND	
2 (18/05/11)	B	Bird 2Bc (dead)	14	Brain: mixed incl Lm on LSA	ND	
2 (18/05/11)	B	Bird 2Bd (dead)	14	Brain: NSO	ND	
2 (18/05/11)	B	Bird 2Be (dead)	14	Liver: NSO	ND	Marked perihepatitis, pericarditis, very poor body condition
2 (18/05/11)	C	Bird 2Cf (live)	1	Brain: NSO	ND	Holding head back following transit during import
Death in transit (02/06/11)	D	Birds x 6 (dead)	1	Organ pool-incl Lm on LSB	ND	

ND, not done; Lm, Listeria monocytogenes; NSO, no significant organism; NG, no growth; LSA, Listeria Selective Agar; LSB, Listeria Selective enrichment Broth;

592 Table 2: Summary of the bacteriological results from environmental samples

Submission	Batch	Sample	Bacteriology - selective Listeria culture
2	C	Transit box 1 – box liner	NSO
2	C	Transit box 2 – box liner	NSO
2		Water – drinking water	NG
2		Pellets	NG
2		Super fine crumbs	NG
Farm- floor swab	A	Shed 8 - affected	mixed incl **Lm** on LSB
Farm- floor swab	A	Shed 10 - affected	NSO
Farm- floor swab	A	Shed 4 - not affected	mixed incl **Lm** on LSB
Farm- floor swab	C	Shed 19 - affected	mixed incl **Lm** on LSB
Farm- floor swab	A	Straw – shed 10 affected	mixed incl **Lm** on LSB
Farm		Clean straw	mixed incl *Listeria innocua* on LSA/ LSB
Farm		Multivitamin solution	NG
Farm		Water	NG

593 NSO, no significant organism; NG, no growth; MG, mixed growth; Lm, *Listeria*
594 *monocytogenes*; LSB, Listeria Selective enrichment Broth; LSA, Listeria Selective Agar
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596
597 **Figure legends:**

598 Fig. 1: Torticollis in a 16-day-old live partridge chick.

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Fig. 2: Brain, parasagittal section, border of brainstem and deep cerebellar white matter of a red-legged partridge: Microabscess with mixed mainly mononuclear leucocyte infiltration with perivascular lymphocytic cuffing, gliosis and central necrosis. Note swollen degenerate axons within vacuoles (arrows) (HE, scale bar = 200µm). *Inset*: Gram positive bacilli (arrows) within the microabscess (Gram stain, scale bar = 20 µm).

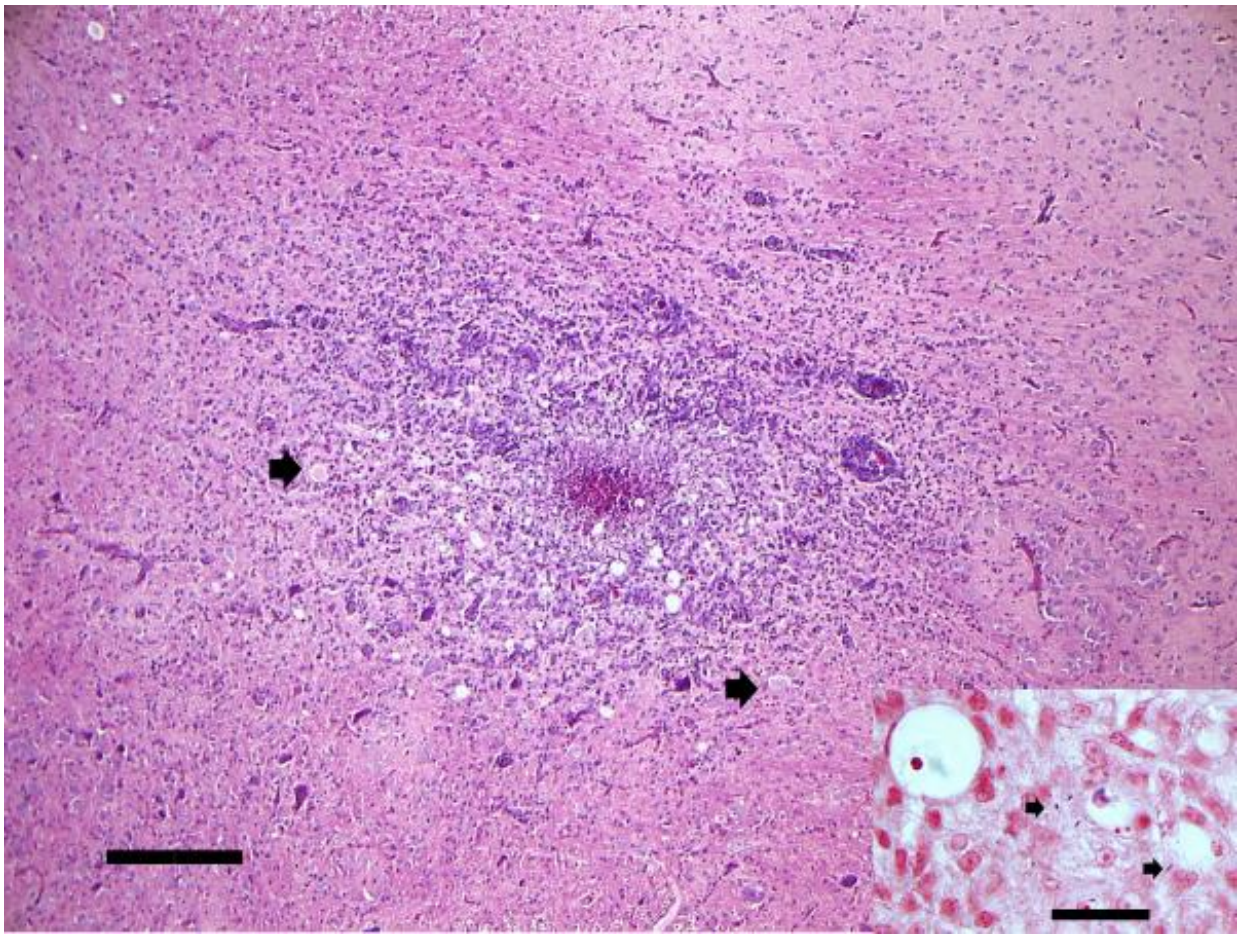

Fig. 3: Phylogenetic analysis of representatives of the genus *Listeria* (Graves et al., 2010) inferred from 16S rRNA gene comparison. The tree was constructed in MEGA5 using the neighbor-joining approach following CLUSTAL alignment of sequences trimmed to a 1397 bp consensus contig and applying the Jukes-Cantor substitution model. Numbers at nodes correspond to proportions of 500 resamplings that support the topology shown with values >50% indicated. Bar = 0.005 substitutions per nucleotide position. The isolate from the case referred to in this report (bird 1Aa) is identified as 19-B006-05-11.

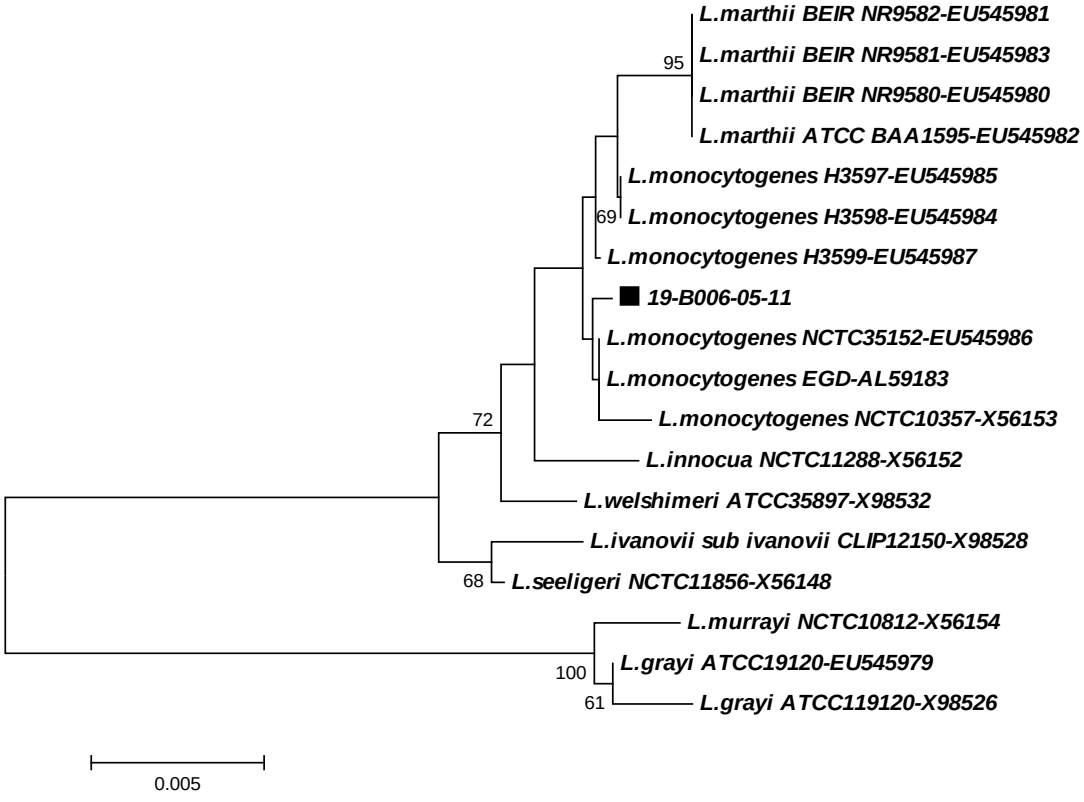

Fig. 4: Phylogenetic analysis of representatives of the genus *Listeria* based on concatenated *sigB, gap* and *prs* sequences showing the position of 19-B006-05-11 relative to sequences described by Graves et al. (2010). The tree was constructed in MEGA5 using the neighbor-joining approach following CLUSTAL alignment of sequences and applying the Jukes-Cantor substitution model. Numbers at nodes correspond to proportions of 500 resamplings that support the topology shown with values >70% indicated. Bar = 0.01 substitutions per nucleotide position.

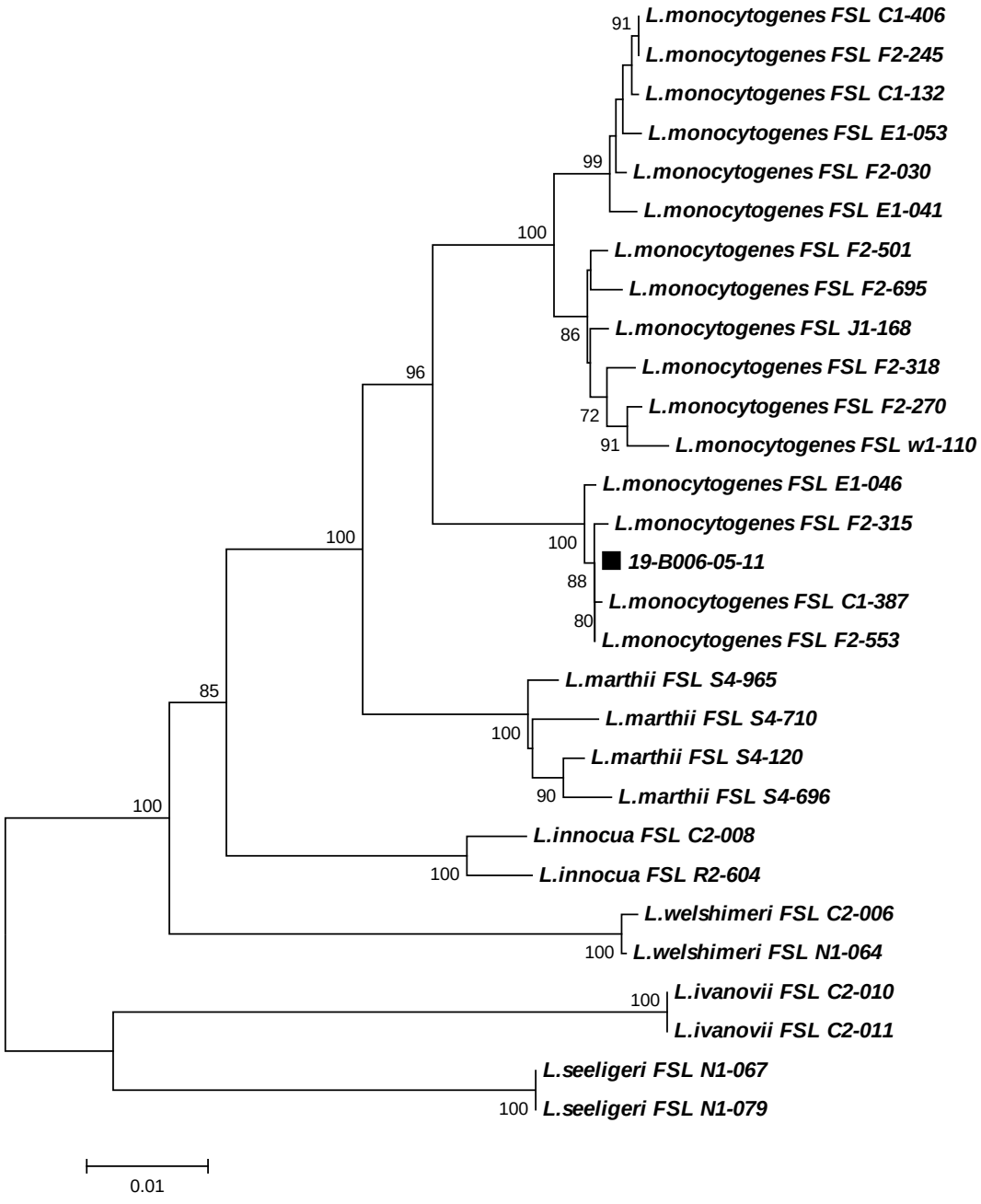

Fig. 5: Dendogram of fAFLP similarity. *L. monocytogenes* isolates of various human and animal sources including isolates from partridges and environment of this outbreak (first five isolates) and one isolate from a partridge from a different case in 1992 (Partridge- heart) underwent serogrouping and fAFLP testing for similarity. The first isolate 'Partridge – brain' refers to the initial isolate (19-B006-05-11) in this outbreak from the brain of the submitted 16-day-old partridge. The second isolate 'Partridge – organ pool' refers to the isolate from the organ pool of one-day-old partridges that had never entered the farm.