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Osteomyelitis in a slaughter turkey flock caused by *Yersinia pseudotuberculosis* sequence type ST42

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ABSTRACT

A *Yersinia pseudotuberculosis* outbreak was diagnosed in a male turkey flock in Finland. *Y. pseudotuberculosis* is a quite rare zoonotic bacterium, which typically causes enteritis in humans and sudden death in animals. In this study, osteomyelitis was diagnosed in small, lame, 11- to 12-wk-old male turkeys. Lameness and slower growth among the turkeys was observed on the farm. During pathological examination, multiple lesions were found in the metaphyseal and physeal areas of the femurs, tibiotarsi, and tarsometatarsi, with multifocal to coalescing mixed heterophilic/granulomatous necrotizing osteomyelitis. *Y. pseudotuberculosis* was isolated from the femoral and tibiotarsal bones or from the joints of six lame turkeys sent for necropsy. The isolation required homogenizing of lesion tissue in phosphate-mannitol-peptone broth, which was cultured directly - and, if needed, after cold enrichment – on selective cefsulodin-irgasan-novobiocin agar. Whole-genome sequencing was used for identification and typing. All isolates belonged to bio/serotype 1/O:1a and sequence type ST42 (Achtman scheme), which is commonly reported in both human and animal *Y. pseudotuberculosis* infections in Europe. The isolates from all six turkeys showed only one to two allele differences in the core genome comparison, indicating a common source of infection. All asymptomatic turkeys were slaughtered at the age of 17 weeks. Whole and partial carcass condemnation rates at the slaughterhouse were high, but no macroscopic changes in the skeletal system were found, showing that food chain information is essential. This study confirms earlier findings that *Y. pseudotuberculosis* can cause osteomyelitis in fattening turkeys, leading to lameness. Food chain information is essential for slaughterhouse operations, to protect the workers and emphasize good working hygiene during slaughter.

1. Introduction

Yersinia pseudotuberculosis is a rod-shaped pathogenic bacterium belonging to the *Yersiniaceae* family of the order *Enterobacteriales* (Adeolu et al., 2016). It is a zoonotic pathogen with animal reservoirs and is typically transmitted fecal-orally through contaminated food or water (Fredriksson-Ahomaa, 2015). All correctly identified *Y. pseudotuberculosis* strains are considered pathogenic, which carry virulence genes located on the virulence plasmid (e.g. *vir*F and *yad*A) and in the chromosome (e.g. *ail* and *irp*) (Fredriksson-Ahomaa et al., 2018). Diarrhoea, abdominal pain, and fever are common symptoms of *Y. pseudotuberculosis* infection in humans. Extra-intestinal symptoms, such as joint pain and skin rash, may occur occasionally, and dissemination of the infection to the blood stream and deep tissue has also been reported (Williamson et al., 2016). A vertebral osteomyelitis caused by *Y. pseudotuberculosis* was fairly recently reported in a patient (Ishihara et al., 2016). However, *Y. pseudotuberculosis* is a quite rare cause of human yersiniosis compared to *Y. enterocolitica,* which is responsible for 99% of all cases (EFSA and ECDC, 2019). Most cases are sporadic, but foodborne outbreaks have also been reported recently in Finland and New Zealand (Williamson et al., 2016; Castro et al., 2019).

Y. pseudotuberculosis has been isolated from a variety of animal species, especially from rodents and birds (Le Guern et al., 2016; Fredriksson-Ahomaa et al., 2018). Animals are usually asymptomatic carriers but acute infection may occur under stress. Avian yersiniosis has been reported in wild and domestic birds (Stoute et al., 2016). The

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infection can range from subclinical to acute disease. Typical lesions include hepatitis, splenitis, nephritis, pneumonia, and enteritis. Osteomyelitis is a rare diagnosis in *Y. pseudotuberculosis* infections. It has been reported in *Y. pseudotuberculosis* outbreaks in turkeys in England and the US, as reported in two older studies (Wise and Uppal, 1972; Wallner-Pendleton and Cooper, 1983) and recently in the UK in a non-human primate with a systemic *Y. pseudotuberculosis* infection (Walker et al., 2018).

Isolating and identifying *Y. pseudotuberculosis* is very demanding (Fredriksson-Ahomaa, 2015). *Y. pseudotuberculosis* is a slow-growing bacterium, which is often overgrown by other bacteria present in the sample. No high-selective enrichment broths or agar plates are available for *Y. pseudotuberculosis* isolation (Fredriksson-Ahomaa, 2015). Furthermore, *Y. pseudotuberculosis* cannot be definitely differentiated from the other *Y. pseudotuberculosis* complex species, i.e. *Y. pestis, Y. similis,* and *Y. wautersii*, using matrix-assisted laser desorption/-ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and phenotypic tests (Fredriksson-Ahomaa et al., 2018). To overcome this problem, genome-based methods, such as multi-locus sequence typing (MLST) based on seven house-keeping genes or core genome MLST (cgMLST), are currently used to an increasing extent for identifying and characterizing bacterial pathogens (Savin et al., 2019).

In this study, we describe a rare outbreak of pathogenic *Y. pseudotuberculosis* causing osteomyelitis in a commercial turkey flock in Finland. We also discuss the *post-mortem* findings at the slaughterhouse. Furthermore, we report the isolation and characterization methods used.

2. Methods

2.1. Case description

In November 2019, 3426 1-d-old turkey poults from a local turkey hatchery were brought to a Finnish commercial turkey farm. At first, the female (n = 1027) and male (n = 2399) poults were reared in one house separated by a fence. The male poults grew slowly and unevenly, and their mortality increased during the first weeks. The culling rate (the sum of sick and dead birds) during the first two weeks was around 4%. The farmer also noted male poults with marked opistotonus and difficulty standing upright. The female poults in the same house had no symptoms, and their culling rate was slightly increased (around 2%). The farmer contacted the healthcare veterinarian, and five 14-d-old male turkeys were culled and sent for a pathological examination at the Finnish Food Authority. The pathological and microbiological examination revealed infections in the brain, lungs, and airsacs of the turkey poults caused by *Aspergillus* spp. The specific origin of the aspergillosis was not found. However, the farmer improved the air quality and litter condition in the turkey house. Birds with the most severe symptoms were culled, as no medication for aspergillosis in turkeys is available. At the age of 4 wks, the male turkeys (n = 2306) were transferred to a separate house in the same building complex. During the whole fattening period, the male turkeys experienced uneven and slower growth. When the male turkeys were 8 wks old, the producer first began noticing smaller, lame birds. At the age of 11 wks, lame birds were clearly visible, the primary production manager of the slaughterhouse visited the farm, and three birds with leg problems were culled and sent for pathological examination at the Finnish Food Authority, where *Yersinia pseudotuberculosis* infection was detected from one turkey. The primary production manager also noticed some lacks in the farm biosecurity: there was a pile of peat and miscellaneous items and equipment beside the farm building. Later additional samples from the flock were sent to the laboratory to confirm yersiniosis. Turkeys with clear symptoms were culled. The flock was not medicated. During the whole fattening period, the culling rate in the male turkey flock was around 12%. The male turkeys were slaughtered at the age of 17 wks.

2.2. Pathological examination and sampling

Altogether 12 lame turkeys were submitted for necropsy. Three turkeys were submitted at the age of 11 wks (first necropsy) and nine turkeys were submitted a week later at the age of 12 wks (second necropsy). The turkeys were culled on the farm and sent to the laboratory as bus cargo. A standard pathological-anatomical examination emphasizing the legs was performed on all the turkeys. Samples for a histopathological examination were collected in buffered formalin from the viscera and leg bones. The bone samples were additionally decalcified. The histological samples were stained with a hematoxylin and eosin stain, and examined under a light microscope. Some distal ends of the femurs with lesions, along with some distal and proximal ends of the tibiotarsi with lesions, were submitted to a bacteriological examination. Samples of altered synovial fluid from the knee joints and heterophilic exudate from a swollen foot were also submitted (Table 1).

2.3. Bacteriological examination

Samples from six turkeys were examined bacteriologically (Table 1). The analyses were started on the same day the necropsy was performed. The joint exudate from the first submitted turkey (Necropsy 1, Turkey ID 302) was studied only using standard direct culturing on blood agar (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood incubated aerobically at 37 °C for 24 and 48 h. The samples from the remaining five birds (Necropsy 2, Turkey IDs 345, 346, 349, 350, and 351) were also directly cultured on fastidious anaerobe agar (FAA, Neogen, Lansing, MI, USA) supplemented with 5% horse blood for anaerobic incubation (at 37 °C for 24 and 48 h) and on selective cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid) incubated aerobically at 30 °C for 24 and 48 h. If the direct culture on CIN agar was negative for *Y. pseudotuberculosis*, a food microbiological isolation method was applied. Tissue material from the lesion (at a ratio of 1:10–1:20) was homogenized in phosphate-mannitol-peptone broth (PMPB) according to the U.S. Food and Drug Administration (FDA) (https://www.fda.gov/food/laboratory-methods-food/bam-chapter-8-yersinia-enterocolitica) by Ultra Turrax ® Tube Drive (IKA Werke, Staufen, Germany) at maximum speed for 2 min, and 100 µl of the homogenate was plated on CIN agar. Additionally, cold enrichment in PMPB at 4 °C for 7 d was performed for one sample, which was negative by direct culturing (Table 2).

2.4. Preliminary identification of Y. pseudotuberculosis

For preliminary identification of *Y. pseudotuberculosis*, several putative colonies from blood agar, FAA, and CIN agar were further cultured on CIN agar. Typical "bull's eye" colonies on CIN were chosen for further identification. The MALDI-TOF MS identification using direct smear was performed by a Maldi Biotyper instrument (Bruker Daltonics GmBH, Germany) with MBT Compass software version 4.1.100 and Bruker MBT Compass database with 8468 entries. The MALDI-TOF MS instrument database of the Finnish Food Authority has been extended by additional *Y. pseudotuberculosis* entries but also with *Y. pestis* and *Y. similis* entries missing from the Bruker MBT Compass database. No *Y. wautersii* was

Table 1
Turkey samples used for bacteriological examination in the first and second necropsy.

Necropsy	Turkey ID	Sample	Isolate ID
First	302	Exudate from tarsometatarso-phalangeal joint	302–1
Second	345	Physeal lesion of distal femur	345–2
	346	Metaphyseal lesion of distal femur	346–10
	349	Metaphyseal lesion of proximal tibiotarsus	349–2
	350	Knee joint capsule	350–3
	351	Metaphyseal lesion of proximal tibiotarsus	531–7

Table 2
Isolation methods used and culture results for *Y. pseudotuberculosis*.

Turkey ID	Direct culture on			Plating on CIN from PMPB[a]	
	Blood agar	FAA[b]	CIN[c]	Directly	Cold enrichment
302	+[d]	NT[e]	NT	NT	NT
345	–	–	–	+	NT
346	–	+	+	+	NT
349	–	–	–	+	NT
350	–	–	–	–	+
351	–	–	–	+	NT

[a] Phosphate-mannitol-peptone broth
[b] Fastidious anaerobe agar
[c] Cefsulodin irgasan novobiocin agar
[d] Y. pseudotuberculosis was isolated
[e] Not tested

included in the database. One isolate per sample analysed by MALDI-TOF MS was further identified by the API20E test (bioMérieux, France). Melibiose and raffinose fermentation and citrate utilization were used for biotyping (Tsubokura and Aleksić, 1995).

2.5. Final species confirmation and characterisation of *Y. pseudotuberculosis* using whole-genome sequencing and MLST

Six isolates, each of which originated from different turkeys (Table 1), were sequenced using Illumina chemistry and a MiSeq benchtop sequencer (Illumina, San Diego, CA, USA). After 18–20 h of growth on blood agar at 30 °C, the DNA was purified using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) with the following minor modifications: after 4 h of cell lysis with lysozyme (20 mg/ml) at 37 °C, 4 µl of RNase A (100 mg/ml) and 20 µl of protein K (20 mg/ml) was added, and the suspension was incubated overnight at 56 °C. DNA was isolated using the automated DNA extraction system QIAcube Connect (Qiagen) and eluted twice (50 µl and 100 µl) using sterile nuclease-free water. The purity of the isolated DNA preparations was analysed using a spectrophotometer (DeNovix, Wilmington, USA) and concentrations were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's protocols. Approximately 250 ng of pure DNA from each isolate was used as a starting material for a paired-end library preparation using an Illumina DNA Prep® Library Preparation Kit. Short reads were assembled *de novo* into contigs using Velvet assembler in Ridom SeqSphere+ software (v5). Species identification was performed *in silico* using SpeciesFinder software (https://cge.cbs.dtu.dk/services/SpeciesFinder/). Serotyping was conducted *in silico* using the software BLAST by aligning the assemblies against 21 different O-antigen sequence clusters of *Y. pseudotuberculosis* (Kenyon et al., 2017). The presence of virulence genes *vir*F and *yad*A, located on the virulence plasmid, the *ail* gene in the chromosome, and the *irp*1 gene in the high-pathogenicity island (HPI), were studied *in silico* by mapping the virulence gene sequences obtained from the virulence gene database VFDB (http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Yersinia) against the assemblies.

Traditional seven-gene MLST based on the Achtman scheme (Laukkanen-Ninios et al., 2011) and the McNally scheme (Hall et al., 2015) were defined using the Yersinia database of the Enterobase platform (https://enterobase.warwick.ac.uk/species/index/yersinia). The schema for an ad hoc cgMLST was defined based on 25 *Y. pseudotuberculosis* O:1 complete genomes available at Genbank using a target definer tool within the Ridom SeqSphere+ software (Ridom, Münster, Germany). In total, 2870 genes were included into the used cgMLST schema. In this study, 31 *Y. pseudotuberculosis* genome sequences of animal origin were included in the cgMLST analysis (Table 3) and a minimum spanning tree (MSP) was constructed to visualize the allelic differences among the isolates and to define clusters. The sequences for samples with isolate codes 8, 10, 11, 19, 22, and 24–27 were obtained from the NCBI database. Four *Y. pseudotuberculosis* ST9 isolates were added to the analyses as a control group (isolate codes 28–31).

Table 3
Y. pseudotuberculosis O:1 isolates originating from animal sources in Finland used for cgMLST.

Isolate code	Origin	Year	Serotype	ST[a]	References
1–6	Turkey (*Meleagris gallopavo*)	2020	O:1a	42	This study
7	Chicken (*Gallus domesticus*)	2014	O:1a	42	Pohjola et al. 2016
8	Jackdaw (*Corvus urbicum*)	1998	O:1a	42	Castro et al. 2019
9	House martin (*Delichon urbicum*)	2015	O:1a	42	Unpublished
10–11	Brown hare (*Lepus europaeus*)	1998, 2000	O:1a	42	Castro et al. 2019
12–16	Brown hare (*Lepus europaeus*)	2015–17	O:1a	42	Unpublished
17	Mountain hare (*Lepus timidus*)	2015	O:1a	42	Unpublished
18–19	Hedgehog (*Erinaceus europaeus*)	1998, 2012	O:1a	42	Unpublished
20–21	Wild boar (*Sus scrofa*)	2016	O:1a	42	Fredriksson-Ahomaa et al. 2020
22	Alpine ibex (*Capra ibex*)	2005	O:1a	42	Castro et al. 2019
23	Cat (*Felis catus*)	2020	O:1a	42	Unpublished
24–27	Cattle (*Bos taurus*)	2014	O:1a	42	Castro et al. 2019
28	Mountain hare (*Lepus timidus*)	2015	O:1b	9	Unpublished
29–31	Pheasant (*Phasianus colchicus*)	2014	O:1b	9	Sauvala et al. 2021

[a] Sequence type according to the MLST scheme of Achtman

2.6. Meat inspection

The food chain information (FCI) and meat inspection (MI) results were collected from the slaughterhouse data. In the FCI, the farmer reported the confirmed aspergillosis and suspected *Yersinia* infection of the flock. Altogether, 2040 male turkeys were brought to the slaughterhouse during two subsequent days. The birds were aged 120 d and 121 d. Good working hygiene was emphasized during slaughtering.

3. Results

3.1. Pathological examination

The main findings of the first and second necropsies were uniform and focused on the long leg bones of the turkeys. An individual usually had multiple lesions. The gross examination of the metaphyseal and physeal regions of the long bones revealed roundish cavities, sized up to 7 mm, filled with grey gelatinous material, and often with a white speck in the centre (Fig. 1). Altogether seven turkeys had these lesions at one or both ends of their femur, tibiotarsus, tarsometatarsus, or on several of these locations. Most turkeys also had metaphyseal and physeal gross lesions indicating developmental bone disorders which were mild in the turkeys with the aforementioned cavities and moderate to severe in the

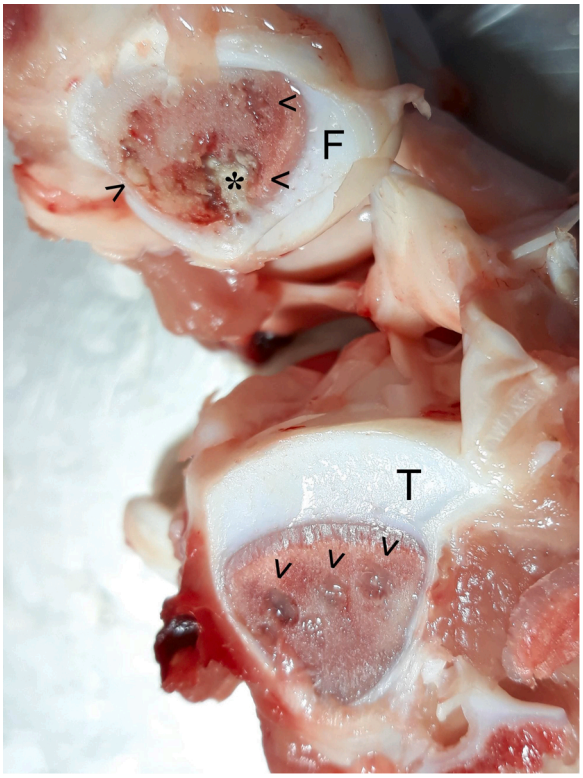

Fig. 1. Multifocal to coalescing chronic severe mixed heterophilic/granulomatous necrotizing osteomyelitis (>) in the distal femur (F) and the proximal tibiotarsus (T) is grossly visible in the metaphyseal and physeal regions as roundish cavities filled with grey gelatinous material. The largest lesion has a piece of necrotic cartilage (*) in the centre.

five turkeys without the cavities. In addition, five turkeys had fibrino-heterophilic arthritis in a knee or foot, five turkeys had heterophilic inflammation in the coilin layer of the ventriculus, three turkeys had chronic airsacculitis, and all turkeys of the second submission had cellulitis in their thighs. In the histopathological examination, the metaphyseal and physeal lesions were diagnosed as a multifocal to coalescing chronic severe mixed heterophilic/granulomatous necrotizing osteomyelitis and as very mild to severe rickets and dyschondroplasia. Rickets and dyschondroplasia were microscopically observable as very mild lesions also in turkeys without any indicating gross lesions.

3.2. Identification and characterisation of *Y. pseudotuberculosis*

Six isolates from six different male turkeys were identified as *Y. pseudotuberculosis* by MALDI-TOF MS scores ≥ 2.3 and an API20E test ID value of 99.8%. All isolates fermented melibiose but not raffinose and did not utilize citrate. Thus, the isolates belonged to biotype 1. Using the whole-genome analyses, all six *Y. pseudotuberculosis* isolates belonged to serotype O:1a based on the O-antigen gene cluster of *Y. pseudotuberculosis* O:1a. They all carried the *lcr*V/*vir*F and *yad*A genes located on the virulence plasmid, the *ail* gene in the chromosome, and the *irp*1 gene in the HPI. Each belonged to the sequence types ST42 and ST96 according to the Achtman and McNally MLST schemes, respectively.

All *Y. pseudotuberculosis* outbreak isolates (1−6) from the turkeys clustered together (Cluster 1), and the largest difference between the neighbouring isolates was not more than two alleles (Fig. 2). Cluster 1 differed clearly from the other ST42 isolates from animals found in Finland (Table 3), where the closest isolate was 337 alleles apart (isolate code 21, wild boar).

3.3. Meat inspection

One turkey died during transport. All remaining turkeys were accepted in the *ante-mortem* examination and proceeded to the *post-*

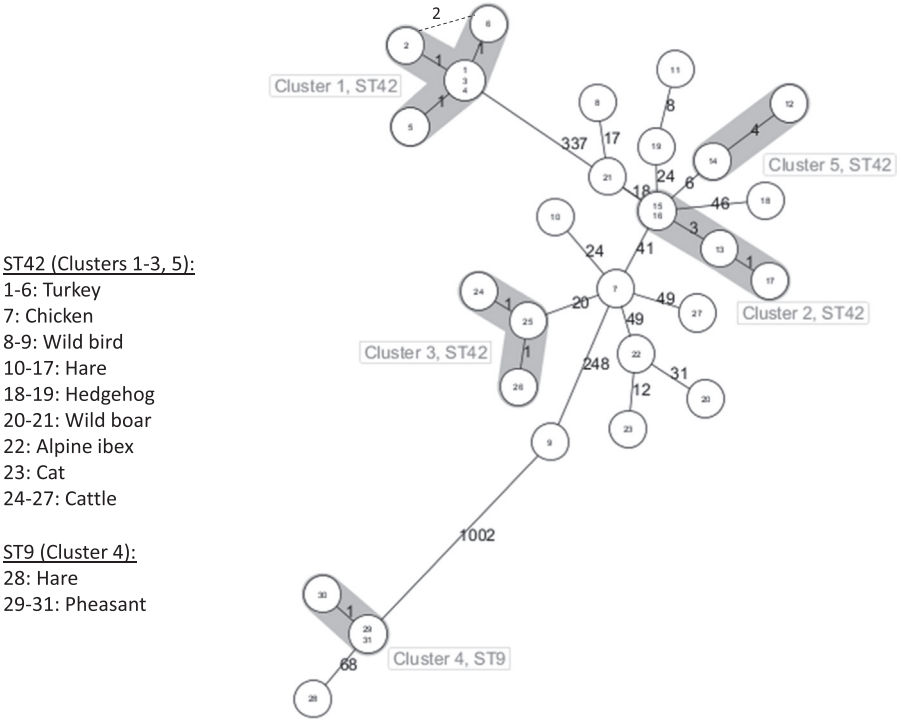

Fig. 2. The minimum spanning tree based on cgMLST allelic profiles of 31 *Y. pseudotuberculosis* including six *Y. pseudotuberculosis* ST42 outbreak isolates found in Finnish fattening turkeys and 25 additional *Y. pseudotuberculosis* ST42 (n = 21) and ST9 (n = 4) isolates from animal sources in Finland.

mortem examination. The mean carcass weight of the turkeys in both slaughter batches was 11.5 kg, varying between 7.5 and 13.9 kg. The whole and partial carcass condemnation rates for the study flock were clearly higher compared to the condemnations rates among all slaughtered turkeys in the same year in Finland (Table 4). Airsacculitis was the most common reason for whole carcass condemnation and skin injuries for partial condemnation in the study flock.

4. Discussion

Y. pseudotuberculosis rarely causes osteomyelitis in animals and humans. In our study, osteomyelitis caused by *Y. pseudotuberculosis* was diagnosed in male turkeys from one fattening flock in Finland. Lameness and uneven growth in the flock was observed, and *Y. pseudotuberculosis* belonging to bio/serotype 1/O:1a was isolated from the bones and joints of 11- to 12-wk-old turkeys. Osteomyelitis in association with *Y. pseudotuberculosis* infection in turkeys has previously been reported in England and the US (California) (Wise and Uppal, 1972; Wallner-Pendleton and Cooper, 1983). In both countries, the affected 12-wk-old turkeys showed severe lameness and low growth rates, which is in accordance with our study (Wise and Uppal, 1972; Wallner-Pendleton and Cooper, 1983). Additionally, turkeys in California showed watery diarrhoea, which was not observed in our turkeys. The culling rate in the Finnish flock was approximately 12%, which was double to the average culling rate of male turkey flocks in the slaughterhouse during the year 2019 according to the primary production manager. In California, morbidity varied between 2% and 15% on three turkey ranches (Wallner-Pendleton and Cooper, 1983) and 25% of the turkeys in England developed clinical signs of severe lameness (Wise and Uppal, 1972). In our study, lameness was probably partly caused by rickets and tibial dyschondroplasia. No mortality was recorded in England but mortality in California was high due to cannibalism. In our study, slower growth and increased mortality (approximately 4%) was detected among 2-wk-old male turkeys, which was most probably due to aspergillosis diagnosed in the flock. Aspergillosis before the *Y. pseudotuberculosis* outbreak may have influenced the severity and morbidity of the yersiniosis. Suffering from aspergillosis may have led to stress in the flock, which could have made the turkeys more vulnerable to *Y. pseudotuberculosis*. The illness caused by these infections may have led to slower growth of the turkeys. High levels of tetracycline in the feed have been shown to arrest yersiniosis; however, surviving medicated turkeys in California were condemned in the *post-mortem* inspection due to visceral lesions (Wallner-Pendleton and Cooper, 1983). No medication was used in the affected turkey flock in Finland. In Finnish poultry meat production, antimicrobials are used first after diagnosis. Until the diagnosis and appropriate medication are determined, the birds with severe symptoms are culled to avoid unnecessary pain and distress to the turkeys. The source of *Y. pseudotuberculosis* infection remained unclear, but we suspect that pest animals such as insects, rodents and birds may have played a role in transmitting the bacteria to the flock by contaminating the feed, drinking water, or litter. The peat pile and unnecessary equipment around the farm building weakened the biosecurity of the study farm by offering shelter and nesting places to pest animals. Laukkanen et al. (2008) have reported that contact with pest animals is associated with a high prevalence of *Y. pseudotuberculosis* on Finnish pig farms.

In the pathological examination, we detected osteomyelitis in the metaphyseal and physeal areas of the femur, tibiotarsus, and tarsometatarsus. Osteomyelitis in the long bones with lesions in or just below the region of the growth cartilages was also reported in England (Wise and Uppal, 1972). Additionally, we found some joint lesions but no typical visceral abnormalities caused by *Y. pseudotuberculosis*, which is in accordance with the English study (Wise and Uppal, 1972). In an experimental inoculation study, granulomatous lesions in the liver and spleen were observed in turkeys infected with high doses of *Y. pseudotuberculosis* (Wise and Uppal, 1972). Osteomyelitic lesions in our study were similar to the gross and microscopic findings in osteomyelitis of turkeys caused by *Staphylococcus aureus* and *Escherichia coli* (Nairn, 1973). Yersiniosis has rarely been reported in turkeys but osteomyelitis appears to be among the most typical diagnosis in *Y. pseudotuberculosis* infections of fattening turkeys (Wise and Uppal, 1972; Wallner-Pendleton and Cooper, 1983).

We isolated *Y. pseudotuberculosis* from affected femoral and tibiotarsal bones and joints. *Y. pseudotuberculosis* has been isolated from bones but not from joints in the aforementioned studies. In our study, isolation was not successful using the standard direct culture method on blood agar and on CIN agar for a majority of the samples. Isolation succeeded by applying a food microbiological method for *Y. pseudotuberculosis* isolation by homogenizing a larger amount of lesion tissue in PMPB before plating on CIN agar. However, one sample required 1 week of cold enrichment in PMPB before *Y. pseudotuberculosis* could be isolated. In earlier studies, lesion samples were plated directly onto blood and/or McConkey agar, which may explain why the pathogen could not be detected in all samples (Wise and Uppal, 1972; Wallner-Pendleton and Cooper, 1983). We recommend taking several samples including bone and joint samples for bacteriological studies and to homogenize lesion material in PMPB before culturing on selective CIN agar. Additionally, cold enrichment in PMPB, which enables the detection of slow-growing *Y. pseudotuberculosis*, should be used if direct culturing of PMPB homogenate is negative.

MALDI-TOF MS was used for preliminary identification of suspected *Yersinia pseudotuberculosis* colonies. All isolates were identified as *Y. pseudotuberculosis*, with high species-level scores indicating that they differed from the highly pathogenic *Y. pestis* and non-pathogenic *Y. similis* included in the library. As the MALDI-TOF MS instrument library did not include *Y. wautersii*, it remained unclear if this missing database entry would have influenced the identification results. The isolates were further identified as *Y. pseudotuberculosis* using the API20E test. Based on the whole-genome sequence results, all isolates belonged to serotype O:1a and carried characteristic virulence genes in the

Table 4
Whole and partial carcass condemnation rates of slaughtered turkeys including main condemnation reasons in the study flock.

Origin	No. of flocks	No. of turkeys	Main condemnation reason	Condemnation % (No. of turkeys)			
				Whole carcass		Partial carcass	
Finland[a]	227	902265		4.8[b]	(43309)	7.5[b]	(153385)
This study	1	2039		17.0[c]	(347)	18.6[c]	(387)
			Airsacculitis	6.5[c]	(133)	1.7[c]	(35)
			Skin injuries	5.0[c]	(102)	14.0[c]	(286)
			Cachexia	2.7[c]	(55)	NR[d]	
			Enlarged sternal bursa	1.5[c]	(30)	0.6[c]	(12)
			Bruise or fracture	0.05[c]	(1)	2.1[c]	(42)

[a] All turkeys slaughtered in Finland in 2019. Data collected by Finnish Food Authority.
[b] Whole and partial condemnation rates among all turkeys in Finland in 2019.
[c] Whole and partial condemnation rates in the study flock.
[d] Not reported.

chromosome and on the virulence plasmid. Identification and serotyping of *Y. pseudotuberculosis* with phenotypic tests have shown to be very challenging and therefore, more accurate genotypic methods are recommended (Fredriksson-Ahomaa et al., 2018). Whole-genome sequencing enables accurate identification and characterisation of *Y. pseudotuberculosis* isolates including serotyping, virulence typing and sequence typing. All these characteristics are important for epidemiological studies.

Y. pseudotuberculosis bio/serotype 1/O:1a was shown to be the cause of an osteomyelitis outbreak in the Finnish turkey flock. No information is available on the serotype or biotype of *Y. pseudotuberculosis* infections among turkeys in earlier studies (Wise and Uppal, 1972; Wallner-Pendleton and Cooper, 1983). *Y. pseudotuberculosis* O:1 has quite recently been reported to have caused osteomyelitis in a lemur in the UK (Walker et al., 2018). Serotype O:1 is the most common type found in human and animal *Y. pseudotuberculosis* infections in Europe (Magistrali et al., 2015; Le Guern et al., 2016). Serotype O:1 also caused the latest reported foodborne outbreaks in Finland and New Zealand (Williamson et al., 2016; Castro et al., 2019).

All outbreak-related isolates belonged to sequence type ST42 according to the Achtman MLST scheme, which is designed for *Y. pseudotuberculosis* strains (Laukkanen-Ninios et al., 2011) and to sequence type ST96 according to the McNally MLST scheme, which is designed for *Yersinia* spp. (Hall et al., 2015). This sequence type has been identified from humans and animals in Europe, including Finland (http://enterobase.warwick.ac.uk/species/index/yersinia). The outbreak isolates studied here were genetically highly similar and differed in their core genome maximally from each other in MST in only two alleles. This strongly indicates a common source. Additionally, the isolates differed clearly from all other *Y. pseudotuberculosis* of animal origin isolated until the present date in Finland.

The meat inspection results of the male turkey flock were poor. The condemnation rates were multiple times higher than the whole carcass and partial condemnation rates of all Finnish slaughter turkeys in 2019 (Finnish Food Authority). Furthermore, the mean carcass weight was low, and the condemnation rate due to cachexia was high, indicating uneven growth at the farm. Airsacculitis was the most common reason for whole carcass condemnation, which was most probably due to chronic aspergillosis (Lair-Fulleringer et al., 2003). Interestingly, no condemnations were made due to arthritis. This may indicate that turkeys with the most severe leg problems had already been culled. Additionally, joint disease and especially osteomyelitis may be difficult to identify at meat inspection. The results of this study demonstrate that identification of *Y. pseudotuberculosis* infection in turkeys during *post-mortem* inspection may be very difficult if no clear macroscopic changes in the bones, viscera, or intestinal track are present. Also, differentiating *Y. pseudotuberculosis* infection macroscopically from other bacterial osteomyelitis may be impossible (Huff et al., 2000). This also emphasizes the importance of FCI, where important information (e.g. growth, untypical behaviour, and laboratory results) from the farm is forwarded to the slaughterhouse before the flock arrives at the slaughterhouse. FCI enables (1) additional measures on working hygiene, (2) changing the slaughtering order, and (3) slowing down the line speed to prevent possible *Y. pseudotuberculosis* infections among workers and to decrease the risk of carcass contamination. In our study, the FCI had a very important role by informing the slaughterhouse and official veterinarians about a flock possibly carrying *Y. pseudotuberculosis*.

5. Conclusions

Y. pseudotuberculosis bio/serotype 1/O:1a with the sequence type ST42 (Achtman MLST scheme) caused a rare outbreak in a flock of male fattening turkeys in Finland. Lameness and uneven growth were observed in the flock. The pathological examination revealed osteomyelitis, and the bacterium was isolated from the bone and joint lesions. Isolation of *Y. pseudotuberculosis* from quickly decomposing avian samples and especially from bone lesions is challenging, and *Y. pseudotuberculosis* may be missed if yersiniosis is not suspected and selective isolation methods are not applied. Lesion tissue material homogenized in PMPB combined with cold enrichment before culturing on selective CIN plates was shown to be a successful method to detect *Y. pseudotuberculosis* from bone lesions. Whole genome sequencing combined with *in silico* analysis is a very accurate way to identify and characterise *Yersinia* isolates. The slaughter results of the remaining turkeys were poor, with high whole and partial carcass condemnation rates. The FCI provided very important information regarding the *Y. pseudotuberculosis*-positive flock to the slaughterhouse. The *Y. pseudotuberculosis* outbreak in the turkey flock caused economic loss due to the large numbers of culled birds and carcass condemnations.

Declaration of Competing Interest

none.

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