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SHORT REPORT
The role of birds in dissemination of *Francisella tularensis*: first direct molecular evidence for bird-to-human transmission

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SUMMARY

During a recent large tularemia outbreak in Bulgaria we found several cases that were remote from the main focus. One case had an unusual mode of transmission. A hunter acquired tularemia through a nail scratch from a buzzard (*Buteo buteo*) and consequently developed a typical ulceroglandular form of the disease. The diagnosis was confirmed by serological methods and successful cultivation. Comparative strain typing was performed by high-resolution multi-locus variable-number tandem repeat analysis (MLVA). The isolated strain was identical to one of the outbreak genotypes. We consider that this case represents a bird-to-human transmission of *F. tularensis*.

Key words: Bird-to-human transmission, *Francisella tularensis*, hunter, MLVA, nail scratch.

Tularemia, also known as rabbit or deerfly fever, is a bacterial zoonosis caused by a small, pleomorphic, Gram-negative coccobacillus, *Francisella tularensis*. It is a severe human pathogen with biological warfare potential [1]. The infection is spread by ticks and aerosol particles. *F. tularensis* has been isolated from rodents, lagomorphs, deer and other ground and water mammals which can explain some water and foodborne outbreaks. Harbouring of *F. tularensis* in protozoa sheds some light on aspects of epidemiology and timing in waterborne outbreaks [2]. The role of birds in dissemination of the disease, however, is not very clear and has often been neglected. Mammals have been considered a preferred host since they have a lower body temperature than birds. In spite of several reports in the literature [3–7], no direct evidence for bird-to-human transmission of *F. tularensis* has been described so far and our observation adds to the body of suggestive evidence supporting this route of transmission.

There have been several outbreaks of tularemia in Bulgaria and we recently published a description of the 1997–2005 outbreak [8]. Five sporadic cases of tularemia were detected along with the main outbreak which appeared distantly from the affected region (89, 98, 105, 110 and 120 km from the epidemic focus). There were no records of previous tularemia incidence in these areas or travel by the infected individuals. We have therefore studied these cases in greater detail; however, controlled reliable information was obtained for only one of the cases. The epidemiological and laboratory findings for this case suggested that bird-to-human tularemia transmission might be involved.

A 33-year-old male hunter presented at the clinic with an enlarged right axillary lymph node. He claimed that, during a pheasant hunt, he was attacked by a buzzard (*Buteo buteo*). While collecting a shot pheasant, he was attacked and his right arm was deeply scratched by the buzzard. The hunter did not

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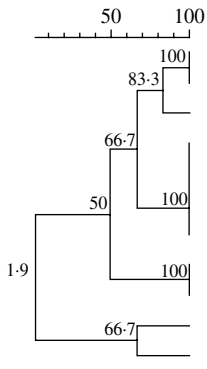

Subspecies	Strain ID	M3	M6	M20	M21	M22	M24
holarctica	Dermacentor-1, Bulgaria 1997, tick	333	374	255	396	254	480
holarctica	Aqua G, Bulgaria 2003, water	333	374	255	396	254	480
holarctica	Mih, Bulgaria 2004, human	342	374	255	396	254	480
holarctica	Gal, Bulgaria 2003, human	288	353	255	396	254	480
holarctica	Drag, Bulgaria 2003, human	288	353	255	396	254	480
holarctica	Hunter, **cultured isolate**	288	353	255	396	254	480
holarctica	Hunter, **lymph node aspirate**	288	353	255	396	254	480
holarctica	L1, Bulgaria 1998, hare	351	311	267	396	254	480
holarctica	Las, Bulgaria 2004, human	351	311	267	396	254	480
tularensis	Schu 4, USA 1941, human	432	311	255	403	242	459
holarctica var. *jap.*	Japan, JPN 1926, human	234	311	436	403	248	459

Fig. 1. Comparison of Bulgarian outbreak isolates and the MLVA pattern obtained with *F. tularensis* DNA amplified from the hunter's lymph node specimen. The corresponding MLVA fragment sizes are in base pairs (bp).

remember any recent tick bites, rabbit hunting, game skinning and handling. He was not involved in handling and cooking the game. Thirty-six hours after the event, he experienced fever, his right axillary area became painful and he palpated an enlarged bump. He was treated by the local general practitioner (GP) with amoxicillin and cephalothin but the symptoms continued. After several visits to other GPs he finally had a serum sample sent to the National Reference Laboratory of High Medical Risk Infections at the National Center for Infectious and Parasitic Diseases (NCIPD) where a positive agglutination titre $>1:2560$ for *F. tularensis* was detected. The hunter was then asked to attend the NCIPD where an aspiration biopsy specimen for culture and PCR analysis were performed.

Axillary lymph node aspirate was obtained by fine-needle biopsy. Bacteriological procedures were carried out as previously described [8]. Briefly, culture isolation was obtained after i.p. inoculation of the aspiration material in mice. DNA was extracted from the *F. tularensis* culture and from 250 μl native aspirate by the standard proteinase K/phenol-chloroform method and stored at $-20\,^{\circ}$C for further genetic analyses [9].

A high-resolution multi-locus variable-number tandem repeat analysis (MLVA) typing system comprising six VNTR loci was applied for comparison of the outbreak isolates and the hunter's isolate and additionally the DNA from the hunter's lymph node aspirate [10]. Some modifications to the MLVA protocol, described by Bystrom *et al.* [10], were made. Briefly, the primers were labelled with Cy5 instead of 6-carboxytetramethylrhodamine for compatibility with ALFexpress II sequencer (GE Healthcare Life Sciences, USA). The primer concentration was adjusted as follows: Ft-M3, 0·12 μM; Ft-M6, 0·15 μM; Ft-M20, 0·05 μM, Ft-M21, 0·06 μM; Ft-M22, 0·03 μM; Ft-M24, 0·5 μM. The reaction volume was 25 μl with the following concentration of PCR components: 100 μM dNTP mix, 2 mM $MgCl_2$, 1$\times$ PCR buffer [50 mM KCl, 20 mM Tris–HCl (pH 8·4), Invitrogen Inc., USA], 5% DMSO (Merck KGaA, Germany), 0·1 μg/μl non-acetylated bovine serum albumin (Sigma-Aldrich, USA), 1 U *Taq* polymerase (Invitrogen). The cycling programme was as described by Johansson *et al.* [11]. Three microlitres of the PCR products were mixed with an equal volume of loading buffer (99·5% deionized formamide, 0·5% blue dextran), denatured for 5 min at 94 °C and loaded on an 8% ReproGel (GE Healthcare Life Sciences). Separation was performed on ALFexpress II DNA sequencer. Electrophoresis conditions were according to the manufacturer's instructions. The gel processing and cluster analysis of the MLVA patterns were performed with Bionumerics version 4.5 (Applied Maths NV, Belgium) software. The dendrogram was generated using categorical coefficient and unweighted pair-group with arithmetic means (UPGMA) algorithms.

The MLVA typing of the isolates obtained from the outbreak focus in Bulgaria revealed four genotypes to be involved (I. N. Ivanov, unpublished observations). These data are in compliance with those of Johansson *et al.* who found that several *F. tularensis* genotypes could be involved in a single tularemia outbreak [11].

Our typing data infer (Fig. 1) that the isolate from the remote tularemia case is identical to one of the four outbreak genotypes. The hunter's isolate could be considered as an outbreak strain transferred to a remote location via the buzzard. Unfortunately no

samples from the bird were available at that time for comparative testing. However, according to the established tularemia surveillance system there have been no human or animal cases registered in the region. Hence it appears that the local origin of the 'hunter strain' in association with existence of enzootic focus is very unlikely.

Comparison of MLVA patterns from the 'hunter strain' and from the native biopsy specimen, revealed 100% identity (Fig. 1). These data suggest that even in the absence of bacteriological isolation MLVA typing could be applied directly on clinical specimens, as shown by others [11].

Another possible route of infection might involve contamination of the scratch wound with pheasant tissue containing *F. tularensis* [6]. However, this hypothesis is less plausible since according to the anamnesis the patient was not involved in game handling and cooking.

The role of birds in the epizoology and dissemination of tularemia is not fully understood, although a number of reports suggesting bird-related transmission have been published in the past century [3–7]. The occurrence of tularemia in birds is of particular interest because they might serve as disseminators of the disease. At least 26 avian species are known to be susceptible to *F. tularensis* infection [4]. Naturally acquired infection seems to occur in gallinaceous birds and data exist for experimental infection in raptors and crowns [3, 5–7]. Ticks (e.g. *Haemaphysalis leporispaulstris*) are the primary source for disease transmission in natural cases of tularemia in upland game birds such as grouse and pheasants [12]. *F. tularensis*-infected ticks transported by migrating birds were believed to be the origin of endemic tularemia in mountain hares (*Lepus timidus*) on an island in the Baltic Sea [13].

It should be noted that the same outbreak MLVA genotype discussed here was also recently reported in Turkey [14]. This similarity between Bulgarian and Turkish outbreak strains was speculated to be of possible relation to migratory bird transfer (or ticks feeding on them) [14].

Birds of prey could be infected and transmit bacteria through feeding of infected prey [15]. Considering the fact that predators catch preferentially diseased animals, one could speculate that selective predation would contribute to bacterial spread [16]. Being relatively resistant to tularemia birds might harbour the microbe for prolonged periods and excrete it in droppings [15, 17].

A possible explanation for the transfer of the outbreak strain to the remote location is that buzzards kill and ingest small rodents infected with tularemia and thereby may become contaminated on their exterior (i.e. nails) or even systemically infected. The described case involves a laceration from a nail scratch that resulted in infection with *F. tularensis*. Being an accident this event has limited epidemiological significance, although it implies some important considerations. First, it suggests that viable and contagious francisellae could be transferred to humans after contact with birds. Second, it proves a link between remote tularemia cases that strongly suggest bird transmission.

Another question that still remains unsolved and which is a possible task for future investigations: 'Is vertical transfer of *F. tularensis* with eggs possible at least in birds that represent asymptomatic carriers?' This question might be of particular epidemiological importance considering that some ground and water rodents feed on eggs.

In conclusion our data suggest that spread of *F. tularensis* by predator birds is possible and humans could be accidentally infected by contact with them. The discussed data should be considered in medical practice and tularemia should be suspected in cases where contact with birds occurs.

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DECLARATION OF INTEREST

None.

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