INVITED REVIEW

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Epidemiological investigation of a tularaemia outbreak after a hare hunt in Bavaria, Germany, 2018

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Summary

In November 2018, a tularaemia outbreak occurred in Bavaria, Germany, among participants of a hare hunt and butchery employees handling the hares. We conducted an epidemiological outbreak investigation, including a retrospective cohort study among hunting participants, to identify likely transmission routes and activities associated with infection. Twelve of 41 participants were antibody-positive for *Francisella (F.) tularensis* (attack rate: 29%). Cases reported influenza-like symptoms ($n = 11$), lymphadenopathy ($n = 1$) and conjunctivitis ($n = 1$). Infection only occurred in those hunting participants present while hares were processed, while risk of infection was highest when directly involved (RR = 10.0; 95%CI: 2.6–392). *F. tularensis* was isolated from 1/4 hares. Only two individuals reported using some of the recommended personal protective equipment (PPE). Occurrence of mainly non-specific symptoms, likely due to early treatment, was not indicative of a specific transmission route. Transmissions via direct (skin/mucosa) contact and by inhalation of contaminated aerosols seem plausible. Promoting and increasing appropriate use of PPE among people processing hares is crucial to prevent future outbreaks.

KEYWORDS
disease outbreaks, *Francisella tularensis*, hares, risk factors, tularaemia, zoonoses

1 | INTRODUCTION

Tularaemia is a bacterial infection caused by *Francisella (F.) tularensis*. The broad host spectrum includes small mammals (hares, rabbits, mice, wildlife and pets), birds and amphibians, while different arthropods mainly play an important role as vectors (deer flies, mosquitoes, ticks) (Hestvik et al., 2015; World Health Organization, 2007). Although fatality through infection with *F. tularensis* ssp. *holarctica*, the only documented cause of human tularaemia in Europe (Maurin & Gyuranecz, 2016), is very low in humans (ECDC, 2017; Kohlmann et al., 2014; Robert Koch Institute, 2016), complications are frequent and may prolong the course of disease (Maurin & Gyuranecz, 2016; World Health Organization, 2007). In most human cases, the incubation period is between 3 and 5 days, although depending on the route and dose of infection, it can range from 1 to 21 days; rarely, it may take up to several weeks (Robert Koch Institute, 2016; World Health Organization, 2007). Early clinical disease mainly presents with non-specific, influenza-like symptoms. Progressed clinical forms depend on the route of infection. Most common are ulceroglandular or glandular (contact to contaminated animal material/water via skin lesions/mucous membrane, stings/bites of infected arthropods), oculoglandular (touching eye after contact to contaminated material/infected animal), oropharyngeal (oral intake of contaminated food/water) and pulmonal/respiratory forms (inhalation of contaminated dust/aerosols). Human-to-human transmission has not yet been described (Robert Koch Institute, 2016; World Health Organization, 2007).

In Germany, the annual incidence is <0.1/cases per 100,000 inhabitants (Faber et al., 2018; Kohlmann et al., 2014). Between 2015 and 2019, 253 cases were reported (annual average 51, range 34–72) (an der Heiden et al., 2019; Beermann et al., 2016, 2017; Robert Koch Institute, 2020; Sin et al., 2018). Nationally reported tularaemia infections are mainly sporadic single cases or small clusters, acquired in Germany (Faber et al., 2018). Known exposure in autochthonous cases could be linked to vectors (ticks or mosquitoes) in some and to hares/rabbits or meat products in most cases (Kohlmann et al., 2014). Most outbreaks in the past were associated with contact to infected hares, including two of the three largest ones in Germany since 2001, affecting between 6 and 11 people respectively. (Burckhardt et al., 2018; Hauri et al., 2010; Sin et al., 2013). Hunters and game butchers, who are frequently in contact with hares, represent a main risk group. The Federal Research Institute for Animal Health recommends avoidance of dust and aerosol formation, the use of gloves, a dust-tight breathing mask and safety goggles when handling game as well as no further disassembling of suspected game (Friedrich-Loeffler-Institut, 2015). The last officially reported fatal tularaemia infection in Germany was in 2017 (Sin et al., 2018). In the German state of Bavaria (~13 million inhabitants), the annual number of reported tularaemia cases ranged from 2 to 13 between 2013 and 2017.

On 5 November 2018, the Bavarian Health and Food Safety Authority (LGL) was informed about eight persons presenting with acute influenza-like symptoms after participating in a hare hunt in Schwandorf county on 27 October 2018. They had presented themselves at the local hospital's emergency department on 4 November 2018, communicated the previous hunt and their suspicion of tularaemia infection.

The local health authority conducted the epidemiological outbreak investigation in cooperation with the LGL, initiated immediate control measures and performed active case finding. Eight hares (*Lepus europaeus*) were shot during the hunt. Four of those were heavily damaged and therefore disposed at a rendering plant. The hunters processed the remaining four carcasses, hung them in a slaughter room at one hunter's home and provided them to a wild game butchery afterwards, where the meat was processed and sold to customers.

We had the opportunity to investigate this outbreak in a timely and detailed manner. Our aim was to assess and describe the extent of the outbreak, identify activities associated with infection and most likely transmission routes. Here, we report the epidemiological outbreak investigation. A manuscript focussing on laboratory testing in this outbreak has been published elsewhere (Jacob et al., 2020).

2 | MATERIAL AND METHODS

2.1 | Study design

All persons with potential exposure to *F. tularensis* during the hunt, through further processing of game or contact to potentially contaminated or infected hunting dogs were contacted by the local health authority and invited to participate in the epidemiological study. Our study included the collection of questionnaire information (self-administered) and at least one blood sample. The simultaneous collection was organized by the local health authority. Aiming to identify risk factors for infection, we conducted a retrospective cohort study including a subset of study participants, namely hunting participants. The investigation was also extended to the participating hunting dogs and hunted hares.

Impacts

- Game (including hares) infected with *Francisella tularensis* poses high risk of infection for persons getting in close contact.
- Early suspicion and rapid treatment and detection of tularaemia infection may help to prevent more severe courses of disease.
- In order to prevent further outbreaks, use of recommended PPE is necessary, which may be targeted through increased awareness of tularaemia infection and potential transmission routes, especially among risk groups handling hares.

2.2 | Study population

We defined a possible case as a participant with no antibodies against *F. tularensis* detected at the last sampling point, sampled at least two weeks after exposure, and who reported any of the following symptoms 1–21 days after exposure: fever, chills, headache, limb pain, sudden sweating episode(s), sore throat, nausea, vomiting, stomach pain, cough, stomatitis, pharyngitis, tonsillitis, difficulties breathing or shortness of breath, pneumonia, conjunctivitis, swelling of the eyelid(s), skin rash, skin inflammation or ulcer, swollen lymph node(s). We defined a confirmed case as a participant with antibodies (IgG, IgM and/or IgA) against *F. tularensis*, indicative of acute infection. We focussed on confirmed cases in the analysis; if not explicitly stated otherwise, 'cases' refer to confirmed cases.

The outbreak investigation was conducted as part of the authoritative, official task of the county health departments and the state health department (LGL), and was therefore exempt from institutional review board approval. In addition, all participants gave written consent for blood sampling and use of questionnaire information. We included all contacted persons who consented to participate in our study. In the outbreak investigation, we address the four identified exposure groups separately (hunting participants, butchery employees, veterinary staff, family members).

2.3 | Questionnaire

We developed a questionnaire for the cohort of hunting participants (Appendix, supplement 1), collecting information on symptoms, details about the hunting event and other risk factors. We adapted the questionnaire for butchery employees, veterinary staff and family members.

2.4 | Clinical information

Self-reported clinical information was complemented by the collaborating local hospital (Klinikum St. Marien, Amberg) with details about medical examinations performed and treatment applied. Eleven of 12 hospitalized participants, including the initial group of hunting participants, were admitted to and treated at the local hospital.

2.5 | Laboratory methods

2.5.1 | Human testing

The collaborating local hospital performed diagnostic tests at their laboratory among admitted persons, using blood cultures, swabs and sera. Additional oropharyngeal swabs were tested for influenza A, B and respiratory syncytial virus (RSV). Sera were tested for *Leptospira* IgG and IgM in an external laboratory using an enzyme-linked immunosorbent assay (ELISA).

Every participant provided at least one blood sample, taken at least two weeks after the initial exposure, allowing this as a minimum time window for antibody development and detection (Jacob et al., 2020; Koskela & Salminen, 1985). The Consiliary Laboratory (CL) for Tularaemia at the Robert Koch Institute (RKI) tested all samples, including those initially tested by the local clinic. The CL used this outbreak as an opportunity to add to the scarce knowledge regarding seroconversion after contact to *F. tularensis*. Therefore, the CL obtained further samples of the initially hospitalized group of hunting participants and closely monitored their serology (Jacob et al., 2020).

For direct detection of *F. tularensis* by specific real-time PCR and inoculation on culture media, the CL used blood cultures (*n* = 56), throat swabs (*n* = 10) and one eye swab (case 12). 76 human blood sera (including repeated samples) were analysed for antibodies against the lipopolysaccharide (LPS) of *F. tularensis*. Serological testing was done by an ELISA for screening of antibodies against *F. tularensis*, and findings were confirmed by Western blot (WB).

2.5.2 | Animal testing

Organs and muscle tissues of four secured hares were analysed at the LGL department of animal pathology and bacteriology and PCR-tested for *F. tularensis* genus and species *holarctica*. Differential diagnosis included cultural and molecular analysis for *Pasteurella*, *Yersinia*, *Leptospira* and *Brucella*. Cultures were further investigated, for example for the identification of the subspecies, at both the LGL and the CL.

Additionally, a local veterinary practice collected samples of involved dogs. The CL tested throat swabs and EDTA blood via specific real-time PCR and serum for the detection of antibodies against *F. tularensis* (Jacob et al., 2020).

2.6 | Statistical methods

The outbreak description included all persons for whom we identified a potential risk of infection and whom we aimed to interview, as well as involved hares and dogs. We displayed the temporal course, including important events of the outbreak and its investigation, and described cases by date of disease onset. We described attack rates by exposure group (hunting participants, butchery employees, veterinary staff and family members).

We conducted risk factor analysis for the cohort of hunting participants. We combined the five activities 'skinning', 'opening up', 'disembowelling', 'rinsing with hose' and 'handling of hares afterwards' as 'processing' of hares. We considered anyone who carried out at least one of those listed activities to have taken part in the processing. We calculated relative risks (RR) with 95% confidence intervals

(CI) using a log-binomial model and taking laboratory confirmed acute infection with *F. tularensis* as outcome of interest. Inclusion of further variables did not yield in an improved multivariable model based on the Bayes information criteria (BIC). As the model with more than one explanatory variable did not perform better than the one with only one explanatory variable, we only report crude risk estimates, in order to avoid overfitting in this small study group.

Due to the small number of cases, we report p-values testing categorical data according to Fisher's exact test. For testing trends using categorical variables, we use an extension of the Wilcoxon rank-sum test. The significance threshold was set at 0.05. We only considered available data for description and analyses. We double entered questionnaire data in EpiInfo (version 4.4.1.0) and performed data analysis in Stata (version 16).

3 | RESULTS

3.1 | Study participants

We identified 42 people with potential exposure to *F. tularensis*. Case 12 belonged to two exposure groups (hunting participants and family members) and is therefore listed separately in both groups throughout (Figure 1). Identified persons invited to participate in the investigation included hunting participants (n = 35), butchery employees who handled the hares after the hunt (n = 4), family members of a hunter's household where hares hung to bleed out (n = 2) and employees of a veterinary practice (n = 2; veterinary assistant and veterinarian) where several involved dogs were tested. Hare parts were sold vacuum packed. All customers were successfully contacted, and the meat was returned before anyone had opened the packaging. Thus, customers who had already purchased parts of the hunted hares were not included in further investigations.

We included 41 of the 42 invited persons (97.6%) in our outbreak investigation (Figure 1); the veterinarian did not participate. Questionnaire and laboratory information was available for all 41 participants; laboratory information was available for all four available hares and ten of the 11 involved dogs. We identified 11 possible and 12 confirmed cases. All confirmed cases reported having had symptoms (Figure 2). First detection of antibodies was two weeks (n = 2), last 21 weeks (n = 1) after exposure (no testing between three and 21 weeks after exposure); most remaining cases were first confirmed following sampling three weeks after exposure (Jacob et al., 2020). Maximum time windows for serological testing after possible exposure among those with no evidence for an infection with *F. tularensis* were 14 days (n = 1), 18 days (n = 1), 19 days (n = 1), 26 days (n = 20), 34 days (n = 2), 37 days (n = 3) and 21 weeks (n = 1).

3.2 | Hunting participants

Of the 35 hunting participants, 21 were hunters, and 14 were beaters. Median age was 28 years (range: 11–65). Ten participants confirmed as cases (attack rate 28.6%).

3.2.1 | Clinical and treatment information

All confirmed cases reported mainly influenza-like symptoms, except case 12 (Table 1). Headache (8/10), limb pain (8/10), chills (7/10) and sudden sweating episode(s) (5/10) were most often reported. A one-sided conjunctivitis (case 12), a pharyngitis and swollen lymph nodes were each reported once. None of the patients reported any
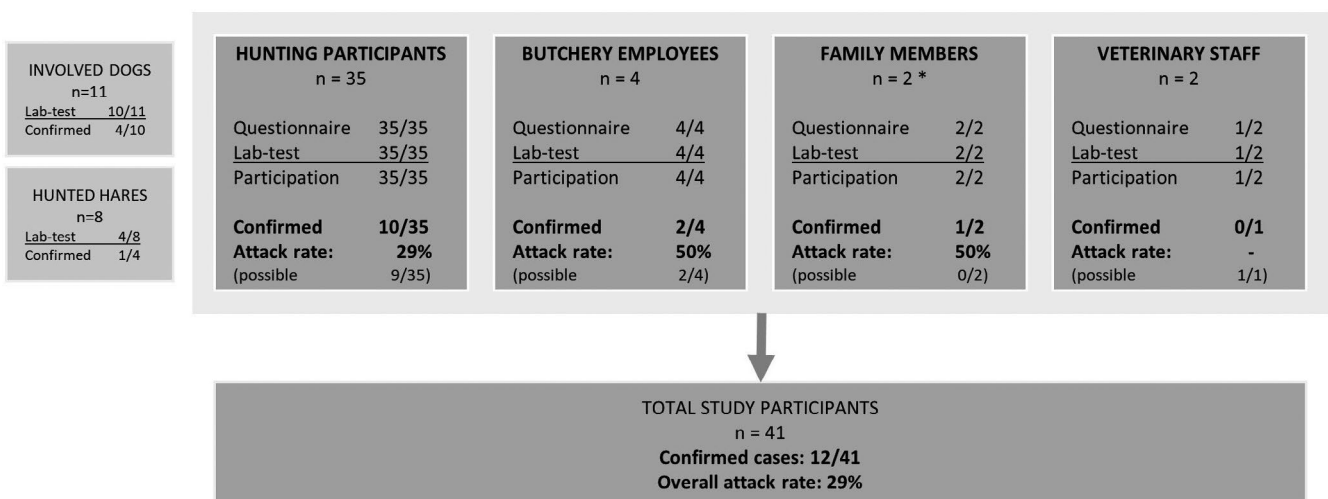
* One case had two potential exposures and is displayed both among hunting participants and family members, but only counted once in the total of study participants

FIGURE 1 Tularaemia outbreak in Bavaria, Germany, 2018: Overview of persons potentially exposed to *Francisella tularensis* following a hare hunt by exposure group, including participation in the investigation (questionnaire and lab-test availability), case definition (possible and confirmed cases) and attack rate
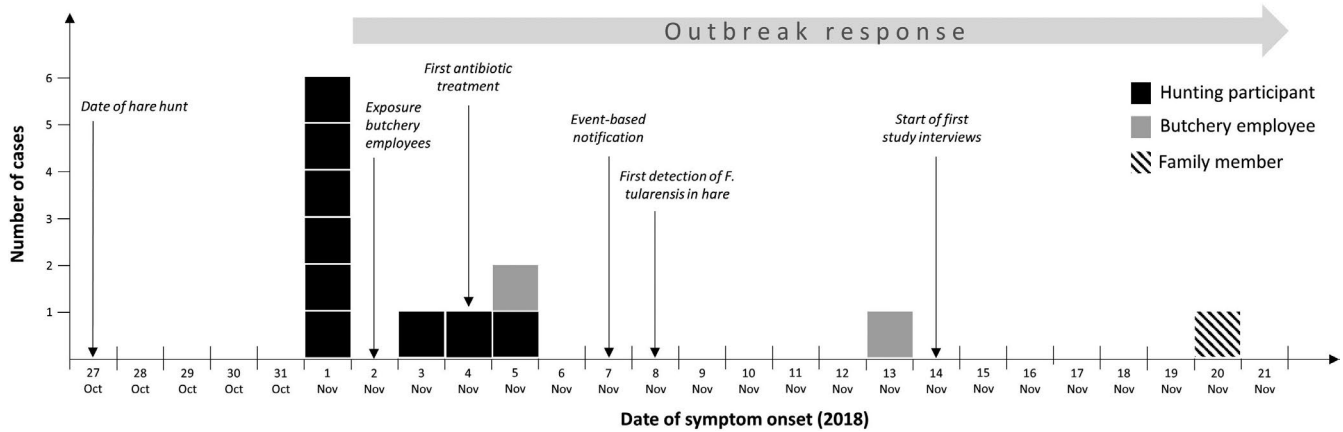
FIGURE 2 Tularaemia outbreak in Bavaria, Germany, 2018: Confirmed tularaemia cases (*n* = 12) by date of symptom onset and time line of associated events

TABLE 1 Exposure and self-reported clinical information of confirmed tularaemia cases, tularaemia outbreak in Bavaria, Germany, 2018

Case	Type of exposure	Disease onset	Symptoms	Antibiotic treatment	Hospitalization
Case 1	Hunt	01.11.2018	Headache, limb pain, sore throat, chills, cough	Yes	Yes
Case 2	Hunt	03.11.2018	Headache, limb pain, fever, chills, sudden sweating episode(s)	Yes	Yes
Case 3	Hunt	01.11.2018	Headache, limb pain, fever, chills, sudden sweating episode(s), cough	Yes	Yes
Case 4	Hunt	01.11.2018	Headache, limb pain, fever, chills, sudden sweating episode(s), nausea, weight loss (5kg)	Yes	Yes
Case 5	Hunt	01.11.2018	Headache, limb pain, fever, chills, sudden sweating episode(s), weight loss (6kg)	Yes	Yes
Case 6	Hunt	05.11.2018	Limb pain, sore throat	Yes	Yes
Case 7	Hunt	01.11.2018	Headache, limb pain, fever, chills, sudden sweating episode(s), pharyngitis	Yes	Yes
Case 8	Game Butchery	05.11.2018	Headache, limb pain, fever, chills, cough, weight loss (1kg)	Yes	Yes
Case 9	Hunt	01.11.2018	Headache, limb pain, chills, weight loss (2kg), swollen lymph node (armpit)	Yes	Yes
Case 10	Hunt	04.11.2018	Headache, sore throat, diarrhoea	Yes	No
Case 11	Game Butchery	13.11.2018	Headache, limb pain, fever, chills, sudden sweating episode(s), diarrhoea, cough, kidney congestion	Yes	No
Case 12	Hunt/Family member	20.11.2018	Swelling of the eyelid (one-sided)	No	No

skin condition, such as skin inflammations or ulcers. The case with latest disease onset (case 12) was potentially exposed later; we address this in more detail under 'Family members'. The hunt, as the day of exposure, took place on October 27, and symptom onsets were reported between November 1 and November 20 (Figure 2). Median incubation period was 5 days (IQR: 5–7; range 5–24 days). All but case 12 reported having consulted a physician and to have received antibiotic treatment.

According to the local hospital, nine hunting participants were admitted to their clinic as a precautionary measure for medical monitoring after the self-reported suspicion of tularaemia

infection. Eight of them confirmed as cases. All nine reported influenza-like symptoms at time of admission, including headache, limb pain, fever and chills, general weakness and reduced general state of health as well as night sweats. Additionally, chest pain was reported in three cases, dizziness and dry cough in one case and painful swelling of lymph nodes in the left upper limb/axilla in one case. No one reported or was clinically diagnosed with ulcers or eschars, stomatitis, tonsillitis, pharyngitis or pneumonia. No gastrointestinal symptoms were reported during their hospital stay. All nine hunters received an electrocardiography (ECG) and a chest X-ray. Chest X-rays did not reveal pulmonary infiltrates. Four

TABLE 2 Information on contact to hares and applied personal protective equipment among 35 hunting participants, tularaemia outbreak in Bavaria, Germany, 2018

Activities with contact to hares	n	Injury during activity	PPE			Cleaning hands		
			Gloves	Goggles / glasses	Mask	Cloth	Water	Water and soap
Direct contact to hare(s) (Touched with hands)	17	NA	1	NA	NA	NA	NA	NA
Carrying of hare(s)	16	NA	0	NA	NA	1	2	0
Emptied bladder of hare	5	0	0	NA	NA	1	1	0
Skinned hare	7	1 (maybe)	1	1	0	0	0	6
Opened up hare	6	0	2	1	0	0	1	4
Disembowelled hare	6	0	2	1	0	0	1	4
Rinsed hare with hose	5	0	2	1	0	NA	NA	NA
Contact after processing of hares (e.g. packing)	2	0	0	1	0	0	2	2

Abbreviation: PPE, personal protective equipment.

hunting participants received an echocardiogram to rule out peri- or perimyocarditis, following non-specific changes in the ECG analyses (mostly changes of the ST segment). Echocardiograms showed normal results in all but one case, for whom changes of the mitral valve unrelated to tularaemia infection were detected; follow-up was recommended. Samples tested negative for all further pathogens considered. All nine received antibiotic treatment with ciprofloxacin 500 mg po bid for 14 days (treatment was continued after inpatient treatment). Clinical course was uneventful in all admitted hunting participants. All were discharged on oral antibiotics after 3 to 5 days.

3.2.2 | Potential exposures and personal protective equipment (PPE)

The attack rate was almost twice as high for hunters (38.1%) compared to beaters (14.3%); however, the RR was not statistically significant (Table 3). The proportion of hunting participants with tularaemia infection increased significantly with the number of hares they were in contact with, from 11.1% (2/18) with no direct contact to 14.3% (1/7) with contact to 1–2 hares and 75% (6/8) with contact to 3–8 hares ($z = 3.10$; $p = .002$). Overall, direct contact to hares was associated with a 4-fold risk of tularaemia infection (RR = 4.24; 95% CI: 1.04–17.18); the risk was almost 7-fold for contact to 3–8 hares compared to no contact (RR = 6.8; 95% CI: 1.7–26.5). Regarding the two cases with no direct contact to hares, case 12 was potentially exposed later.

Three of the four participants who reported injuries (e.g. scratches, open areas) on hands or forearms at the time of the hare hunt became cases; none reported skin inflammations or ulcers. Seven out of 25 who could not remember or negated injuries were case. Injuries were associated with infection (RR = 3.3; 95%CI: 1.4–7.9).

Participants directly involved in the processing of hares had a 10-fold risk of tularaemia infection (RR = 10.0; 95%CI: 2.6–39.2). No one involved in the processing reported injuring themselves during these activities (0/10); eight became cases (attack rate 80%).

Two participants stated wearing gloves during processing activities (one case). Several participants used a cloth, water or water and soap to clean their hands following activities with direct hare contact (Table 2). One person who was involved in the processing of hares and tested negative had worn glasses (any type); no one had worn a mask.

Apart from those hunting participants directly involved in the processing of hares, 11 further participants were present, of whom two became cases, among those also case 12. Of the 14 remaining participants who were not present at the time of hare processing, none became a case. Of those, four reported direct contact to hares; all had carried them, during which one had worn gloves; one had additionally emptied the bladder without wearing gloves.

Eight of the ten dogs that joined the hunt had hare contact. Eight participants had subsequent contact to dog saliva or rabbit blood attached to the dog; four of them became cases. Three of the four had also been directly involved in the processing of hares, whereas the fourth, case 12, was only present during the processing.

None of the cases reported having been exposed to or having noticed other known risk factors for tularaemia infection during the hunt (e.g. contaminated dust/water, tick/insect bite).

3.3 | Butchery employees

Three butchery employees reported hare contact (e.g. touched, washed, disassembled); one person was not sure. However, all four specified the days of hare contact and the activities they had carried out. No one had worn PPE during any of the processing activities (Table 4). Two butchery employees tested positive (attack rate 50%).

3.3.1 | Clinical and treatment information

All four had contact to hares on November 2, and employee 1 had additionally contact on October 30 (Table 4). Disease onsets were on November 5 and November 13, whereby the incubation period was 3 and 11 days respectively. The two cases reported influenza-like symptoms (Table 1). Both stated having had a headache, limb pain, fever, chills and cough. Skin inflammations or ulcers were not reported. Both cases stated having received antibiotic treatment, and one stated to have been hospitalized.

According the local hospital's records, two butchery employees were admitted as a precautionary measure for monitoring purposes after self-reporting their suspicion of tularaemia infection. In accordance, tularaemia infection was confirmed in one of them. They reported influenza-like symptoms on admission including fever and chills, limb pain and reduced general state of health. Ulcers or eschars, stomatitis, tonsillitis, pharyngitis or pneumonia were not reported or clinically observed. One employee received an ECG and a chest X-ray that were both unremarkable. Samples tested negative for all further pathogens considered. Both received antibiotic treatment with ciprofloxacin 500 mg po bid for 14 days (treatment was continued after inpatient treatment). Clinical course was uneventful, and both patients were discharged after 2 and 3 days of inpatient treatment respectively.

3.4 | Family members

The four hare carcasses hung in the slaughter room of a hunter's home overnight to bleed out before providing them to the game butchery. The hunter himself, who was also involved in the processing of hares, confirmed as a case. One of the two family dogs had participated in the hunt, whereas both dogs had licked hare blood in the slaughter room. Two further family members, of whom one had also participated in the hunt as a beater and confirmed as a case (case 12), did not report any direct contact to the hares or hare blood, neither during the hunt nor at home, but reported frequent contact to the dogs. Case 12 developed a conjunctivitis on one eye on November 20, as the only sign of infection. The other family member who did not participate in the hunt tested negative.

3.5 | Veterinary staff

As a precaution, ten of the 11 involved dogs were tested. Staff of the veterinary practice was informed about the tularaemia suspicion beforehand. One veterinarian and one veterinary assistant treated the dogs. Both used PPE during all procedures, including facemask, gloves and surgical gown. General examination and blood sample collection was done between November 6 and 14.

The veterinary assistant reported multiple symptoms, starting on November 11. Tularaemia suspicion was not confirmed, and her

test revealed a negative result for antibodies against *F. tularensis*; symptoms could be attributed to another cause.

3.6 | Animal testing

3.6.1 | Hare samples

The LGL laboratory isolated *F. tularensis* from an abdominal lymph node of one hare. Results were confirmed by specific PCR at the CL; *F. tularensis* subspecies *holarctica* was identified through culture. Details on laboratory findings and phylogenetic analysis of the *F. tularensis* strain are described elsewhere (Jacob et al., 2020).

3.6.2 | Dog samples

None of the dogs who had potential contact to the hares or their body fluids showed any symptoms. Culture and PCR testing of all 10 tested dogs revealed negative results. Serological testing provided tularaemia specific IgG antibody detection in four dogs, with no increase in paired samples within eight days.

4 | DISCUSSION

Comprising 12 cases following a hare hunt in Bavaria in October 2018, we report one of the largest outbreaks of tularaemia attributable to a point source in Germany (an der Heiden et al., 2019; Faber et al., 2018; Sin et al., 2018). Main risk factors for acquiring tularaemia infection in hunting participants were activities with close contact to hares. Risk of infection increased with the number of hares touched; it was highest among those with direct involvement in the processing of hares.

All confirmed cases reported having had symptoms. The majority showed non-specific, influenza-like symptoms. Approximately 15% of tularaemia cases notified in Germany between 2002 and 2016 showed non-specific symptoms, for example fever (Faber et al., 2018). This difference to our observation may be both due to younger age of affected persons compared to other findings (Hauri et al., 2010; Mailles & Vaillant, 2014) and due to the early communicated suspicion of tularaemia infection shortly after symptom onset. Thus, antibiotics specifically recommended for treatment of tularaemia infections and specific testing were immediately initiated. In this outbreak, all hospitalizations among confirmed cases were precautionary after self-reported risk of a potential infection. Clinical courses observed were uneventful. Thus, hospitalization may not be taken as a proxy for severity of infection in these cases. It may be assumed that—if the disease had remained undiagnosed and untreated for longer—cases would have developed more specific and potentially more severe symptoms. Literature of former tularaemia infections with delayed diagnosis and appropriate treatment reported

TABLE 3 Relative risks of tularaemia infection by selected characteristics, cohort study among hunting participants in Bavaria, 2018

Variables	Exposed cases/total	Non-exposed cases/total	Crude RR [95% CI]	p-value
Role as hunter (versus. beater)	8/21	2/14	2.67 [0.66 – 10.75]	.168
Prior injury hand/forearm (versus. no & not known)	3/4	7/31	3.32 [1.40 – 7.87]	.006
Hare contact of participating hunting dog	4/8	0/4	-	-
Direct contact to hare(s) (Touched with hands)	8/17	2/18	4.24 [1.04 – 17.18]	.043
Direct contact to 1–2 hares (versus. no direct contact to hares)	1/7	2/18	1.29 [0.14 – 12.03]	.826
Direct contact to 3–8 hares (versus. no direct contact to hares)	6/8	2/18	6.75 [1.72 – 26.47]	.006
Carrying of hare(s)	7/16	3/19	2.77 [0.85 – 9.00]	.090
Emptied bladder of hare	3/5	7/30	2.57 [0.98 – 6.76]	.055
Present (with no direct involvement) when hares were processed	10/21	0/14	-	-
Directly involved in processing of hares	8/10	2/25	10.0 [2.55 – 39.16]	.001
Skinned hare	5/7	5/28	4.0 [1.59 – 10.06]	.003
Opened up hare	4/6	6/29	3.22 [1.30 – 8.00]	.012
Disembowelled hare	4/6	6/29	3.22 [1.30 – 8.00]	.012
Rinsed hare with hose	4/5	6/30	4.0 [1.73 – 9.26]	.001
Contact after processing of hares (e.g. packing)	2/2	8/33	-	-
Distance ≤5 m while hares were processed	10/20	0/1	-	-
Distance <2 m while hares were processed	9/17	1/4	2.12 [0.37 – 12.25]	.402
Blood splashing while hares were processed (versus. no & not known)	8/10	2/9	3.60 [1.02 – 12.70]	.046
Personal protective equipment				
Wearing gloves during some activities	1/2	9/33	1.83 [0.41 – 8.16]	.426
Wearing glasses during processing of hares	0/1	10/18	-	-
Other known risk factors				
Dust (versus. no & not known)	0/3	10/32	-	-
Surface water (versus. no & not known)	0/7	10/28	-	-
Tick/insect bite (versus. no & not known)	0/2	10/33	-	-

Abbreviations: CI, Confidence interval; RR, Relative risk.

progressed symptoms and more severe courses of disease (Boone et al., 2015; Robert Koch Institute, 2015).

Since mainly non-specific symptoms occurred in this outbreak, clinical courses were not indicative of a specific transmission route. Based on our findings, both transmission through direct skin/mucosa contact and/or inhalation of contaminated aerosols seem plausible. No skin inflammations or ulcers were self-reported or clinically observed among this highly alerted group. Despite early appropriate treatment, small ulcers would have been expected at an early stage of infection after entry of bacteria through small openings in the skin. Previous hand or forearm injury and direct hare contact, however, were significantly associated with infection in the crude analysis. Although small skin lesions may have remained unnoticed, complete absence of such, despite the lack of PPE use, indicates transmission might have been partly airborne, via inhalation of contaminated aerosols produced during hare processing (especially rinsing with a hose). Hauri and colleagues came to a

similar conclusion in their investigation of another tularaemia outbreak among hunters (Hauri et al., 2010). Infectious aerosols generated by washing contaminated products were also responsible for tularaemia outbreaks in sugar beet factories (Puntigam, 1960). In our cohort study, for one of the two cases with no direct contact to hares, no alternative explanation is available for infection, other than through inhalation.

The other case was a hunter's family member, whose property was used to hang hares to bleed out. Although this family member also took part in the hunt as a beater, the case had no direct hare contact, but reported close contact to the two family dogs, which both had licked hare blood and tested positive for IgG antibodies, although the results were indicative of a past infection. Still, transmission could have been mediated through the dogs. This is supported by the considerably later disease onset in this case and the oculoglandular manifestation, which is indicative of a smear infection and could have likely occurred by pathogen

TABLE 4 Exposure information of butchery employees participating in the tularaemia outbreak investigation, tularaemia outbreak in Bavaria, Germany, 2018

Butchery employee	Day of exposure	Touched hare(s)	Activities carried out	Use of gloves	Use of safety goggles	Use of mask	Injuries at time of hare contact	Confirmed case	Disease onset
Employee 1	30.10.2018 02.11.2018	Yes	Disassembled Disassembled	No	No	No	No	No	06.11.2018
Employee 2	02.11.2018	Yes	Vacuum packed	Not sure	No	No	No	Yes	13.11.2018
Employee 3	02.11.2018	Not sure	Put away washed hares	No	No	No	No	No	-
Employee 4	02.11.2018	Yes	Touched	No	No	No	No	Yes	05.11.2018

transmission from a dog via the patient's hand to the patient's eye. Various possibilities of dog-related transmission of tularaemia have been described, both direct and indirect, including after mouthing infected animal carcasses, bringing infected ticks into the home or aerosolizing contaminated particles by shaking or during shearing (Kwit et al., 2019).

None of the participants had used the full set of recommended PPE. Use of PPE was inadequate, among both hunting participants and butchery employees (Friedrich-Loeffler-Institut, 2015). Only two participants stated having used gloves during some activities involving direct hare contact, of whom one tested negative. Nowadays, a hunter's licence in Germany includes training on zoonosis and use of PPE. In this outbreak, however, mainly young hunters who have completed this training were affected. Although reasons for low adherence to current PPE recommendations were not part of the investigation, based on the context, we conclude reasons may have been a lack of adherence to the guidelines rather than lack of knowledge. In absence of available research in this field, reasons for the lack of adherence are purely speculative, but could possibly be due to poor acceptance, low risk assessment or more cumbersome handling when applying these prevention measures. These issues may be worth addressing in future studies.

A strength was the rapidity with which it was possible to respond to the outbreak, conduct the investigation and take appropriate actions, possible through the early tularaemia suspicion raised by one hunter. Prompt measures led to complete identification of involved persons and recall of all sold hare products, which prevented further exposure to *F. tularensis* in customers, thus, potentially preventing further cases. It further enabled rapid treatment and testing, which may have prevented more severe or prolonged courses of infection. Furthermore, it made this extensive and timely investigation possible. There was great willingness for participation—only one person with potential exposure did not participate—resulting in complete questionnaire and laboratory information of all participants. Little time lag between the event and the investigation minimized the risk of recall bias.

For serological testing, a special focus was put on the initially hospitalized hunting participants, who were tested multiple times to closely monitor serology, revealing a late immune response in one of those cases when tested again 21 weeks after exposure (Jacob et al., 2020). This suggests that a limited follow-up time to test for antibodies against *F. tularensis* may miss late seroconversion in some persons. Thus, although testing of most individuals without serological evidence of infection with *F. tularensis* was performed at least two weeks following potential exposures and in most considerably later than that, we cannot rule out that participants who initially tested negative may have seroconverted later and would have been detected in case of prolonged follow-up. This important finding may help guide clinical practice as well future studies on tularaemia. A further limitation was the lacking possibility to analyse the effect of some variables for which we only had few observations and could therefore not conduct a multivariable model.

5 | CONCLUSION

Since cases did not use adequate PPE and in absence of specific clinical presentations of infection, both transmission by direct contact and/or inhalation of infectious aerosols seem plausible. This outbreak clearly shows that close contact to infected game, especially during processing, poses high risk of infection. Early treatment appears to have resulted in mild symptoms in all cases. Our observation underlines the importance of using adequate PPE in adherence to current recommendations among populations at higher risk for a tularaemia infection. Increasing awareness of persons handling and processing hunted hares by informing about the disease, its transmission paths and the importance of PPE use may help prevent future cases and outbreaks. Awareness campaigns in cooperation with relevant professional associations (hunters' or butchers' association) or specialist media (hunting magazines, hunting websites etc.) tailored to these particular risk groups may help tackle the issue of guideline adherence.

CONFLICTS OF INTEREST
None.

DATA AVAILABILITY STATEMENT
Research data are not shared.

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