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# MULTIPLE EPIDEMICS IN AUSTRIAN FRINGILLIDAE CAUSED BY A SINGLE VARIANT OF *SALMONELLA* TYPHIMURIUM

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ABSTRACT: In Austria, numerous deaths of wild birds of the order Passeriformes, family Fringillidae, occurred during the winter months of 2010 and 2012. The Eurasian Siskin (*Carduelis spinus*) was the species most often affected. The dead birds were mainly found in the immediate vicinity of feeding places. Vigilant citizens sent birds ($n$=34) for pathologic examination to the Institute of Pathology in Vienna, Austria. All birds were cachectic or in a very poor nutritional condition. At gross examination, the most striking findings were multifocal to confluent, yellow-white nodules in the crop or esophageal mucosa. In histologically examined birds ($n$=24), severe transmural fibrino-purulent to necrotizing ingluviitis or esophagitis with large amounts of intralesional bacteria was observed. Bacteriologic examination of crop, liver, or other organs from 14 birds revealed abundant growth of *Salmonella* serovar Typhimurium (antigenic formula 1,4,5,12:i:1,2; phage type U277) in all individuals. By means of immunohistochemistry, these bacteria were detected not only in crop mucosa but also in lung tissue. In 17 birds (71%), structures morphologically resembling *Macrorhabdus ornithogaster* were detected histologically on the surface of the proventricular epithelium. Thus, the cause of mass mortality of the passerine birds was identified as infection with *Salmonella* Typhimurium, which was associated with growth of presumed *M. ornithogaster* in many cases.

*Key words:* Austria, Eurasian Siskins, Fringillidae, *Macrorhabdus ornithogaster*, *Salmonella* Typhimurium, wild birds.

## INTRODUCTION

*Salmonella* is the causative agent of salmonellosis and one of the world's most important bacterial infectious agents in humans and animals. These bacteria occur in a large number of serovars and strains, and many of them are zoonotic. *Salmonella* are rod-shaped, usually motile, and 2–5 µm long. They live in the intestine of animals and humans but are also viable for weeks outside the body. Epizootics in wild passerine birds caused by *Salmonella* serovar Typhimurium have been observed repeatedly during the winter months in temperate climate zones. Most frequently, the disease occurs sporadically in the immediate vicinity of feeding grounds, but there are also a number of descriptions of large-scale outbreaks from Great Britain, Norway, Sweden, Poland, Switzerland, Germany, Canada, US, Japan, and New Zealand (Hudson and Tudor 1957; Schaal and Ernst 1967; Wilson and Macdonald 1967; Locke et al. 1973; Hurvell and Jevring 1974; Kapperud et al. 1998; Daoust et al. 2000; Tauni and Osterlund 2000; Alley et al. 2002; Refsum et al. 2003; Hall and Saito 2008; Une et al. 2008; Lawson et al. 2010; Pennycott et al. 2010; Hernandez et al. 2012; Giovannini et al. 2013; Fukui et al. 2014; Krawiec et al. 2014). All bird species are susceptible to *Salmonella* infections, but disease outbreaks are predominantly observed in passerines. This selective vulnerability is not well understood but appears to be dependent on host-related factors, such as bird species, age, and concurrent stress, and on microbe-related factors, such as serovar and strain virulence.

In Austria, several episodes of *Salmonella*-related deaths of wild birds of the order Passeriformes, family Fringillidae, occurred during the winter months in 2010 and in 2012. Such episodes had not previously been recorded in Austria. Our study aimed to report epidemiologic data, pathologic lesions, and results of bacteriologic examination, as

well as to evaluate whether immunohistochemistry is a suitable supportive diagnostic method. Particular aspects of species and sex predisposition, comorbidities, and the involved serovar were considered.

## MATERIALS AND METHODS

### Data collection and postmortem examination

The sudden onset and the large extent of avian deaths in 2010 and 2012 generated much public attention, which resulted in submission of bird carcasses by members of the general public and ornithologists, with the request to determine the cause of death. The date and geographical location of carcass detection and usually observed clinical signs were recorded. In addition, other information, such as duration of the mortality episode and approximate numbers of dead birds that were not submitted, were recorded. A total of 34 songbirds received during 2010 and 2012 were subjected to different examinations. The necropsy protocol always included recording species, sex, weight, body condition, age, and macroscopic findings. From all carcasses with a good state of preservation, a standard set of tissue samples (brain, lung, heart, liver, spleen, kidney, crop, proventriculus, ventriculus, and intestine) was collected for histologic examination.

### Histologic and immunohistochemical examinations

Tissue samples were fixed in 4% buffered formaldehyde solution (10% formalin) for 24 h, dehydrated, and embedded in paraffin wax. Paraffin sections (3–4 μm) were cut and stained with H&E. In cases in which histologic examination revealed structures suspiciously resembling fungal elements, Grocott's methenamine silver stain of the proventriculus and ventriculus was performed.

For specific demonstration of *Salmonella*, immunohistochemistry (IHC) using an anti-*Salmonella* Typhimurium mouse monoclonal antibody (Abexxa Ltd., Cambridge, UK) was applied. Investigated samples were esophageal or crop tissues of 17 cases with characteristic lesions. In nine of these cases, lungs were examined as well, to investigate possible spread of the bacteria to other organs. In two cases, only lung tissue was examined. In five cases, IHC was not done (Table 1). Immunohistochemical investigations were performed using the horseradish peroxidase polymer method on a Lab Vision-Autostainer (Thermo Fisher Scientific, Fremont, California, USA). Formalin-fixed, paraffin-embedded tissue samples were sectioned (3–4 μm) and were placed on positively charged glass slides (Superfrost Plus, Menzel Glaeser, Braunschweig, Germany). After dewaxing and rehydration, antigen retrieval was performed by heating the slides in citrate buffer (pH 6.0) in the Lab Vision PT Module (Thermo Fisher Scientific). The slides were then incubated in Hydrogen Peroxidase Block (Thermo Fisher Scientific) for 5 min, followed by 10-min of incubation in Ultravision Protein Block (Thermo Fisher Scientific), to diminish nonspecific background staining. Sections were then incubated with the primary antibody (dilution 1:100) for 60 min at room temperature, followed by the Primary Antibody Enhancer (Thermo Fisher Scientific) for 15 min, and finally, by UltraVision Large Volume Detection System HRP Polymer (Thermo Fisher Scientific) for 20 min. The signal was visualized with diaminobenzidine Quanto Substrate System (Thermo Fisher Scientific) for 5 min. After the staining reaction, the slides were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany) and mounted with Neomount (Merck) for microscopic examination. To ensure specificity and to rule out potential cross-reactivity with other infectious agents, histologic slides positive for other bacteria (*Escherichia coli*, *Pasteurella multocida*, *Bordetella bronchiseptica*, and *Mycoplasma hyopneumoniae*) or the protozoon *Trichomonas gallinae* were examined, all with negative results. Positive controls were *Salmonella* Typhimurium colonies cut out from agar plates and processed to histologic slides. For negative controls, the specific antibody was replaced with the respective immunoglobulin isotype.

### PCR for *Trichomonas* spp.

To exclude an infection with *Trichomonas* spp., in all cases, a PCR was performed as previously described (Mostegl et al. 2012).

### Bacteriologic examination

From 14 birds, tissues from the crop or esophagus (eight cases with typical crop lesions), liver tissues (five cases without typical crop changes), and tissues from liver, heart, kidney, eye, and eyelid (one case without crop lesions) were collected for bacteriologic examinations, including standard cultivation procedures, followed by biochemical identification of bacterial isolates. For enrichment and selective isolation of *Salmonella*, tissue homogenates were streaked onto xylose-lysine-deoxycholate (XLD) agar (Difco™, BD Diagnostics, Vienna, Austria) as well as transferred into BPW (buffered peptone water; Millipore™, Merck), both incubated at 37 C in ambient air for 24 h. After incubation, 100 μL of BPW cultures were transferred into Selenite and Rappaport-Vassiliadis R10 broth (Difco, BD Diagnostics), incubated at 42 C for 24 h and

TABLE 1. Postmortem findings and results of laboratory examinations of 24 adult wild birds of the order Passeriformes, family Fringillidae, found in the winter months 2010 and 2012 in Austria.[a]

| Species | Location, federal state (city) | Month and year | Sex | Macroscopic crop lesion | Fungus GIT | *Salmonella* serotype | Samples investigated bacteriologically | Phage type | Sepsis[b] | IHC localization |
|---|---|---|---|---|---|---|---|---|---|---|
| Eurasian Siskin | Styria (Rottenmann) | January 2010 | F | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Styria (Rottenmann) | January 2010 | M | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Styria (Rottenmann) | January 2010 | M | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Styria (Rottenmann) | January 2010 | M | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Styria (Irdning) | January 2010 | M | + | + | ND | ND | ND | + | ND |
| Eurasian Siskin | Styria (Irdning) | January 2010 | M | + | + | ND | ND | ND | + | + crop |
| Eurasian Siskin | Styria (Irdning) | January 2010 | M | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Styria (Irdning) | January 2010 | M | + | + | ND | ND | ND | 0 | + crop |
| Bullfinch | Styria (Liezen) | February 2010 | M | + | + | 1,4,5,12:i:1,2 | crop | U277 | + | + crop/+ lung |
| Bullfinch | Styria (Liezen) | February 2010 | M | + | 0 | 1,4,5,12:i:1,2 | crop | U277 | 0 | + crop |
| Bullfinch | Styria (Liezen) | February 2010 | M | + | 0 | 1,4,5,12:i:1,2 | crop | U277 | 0 | + crop |
| Eurasian Siskin | Styria (Donnersbach) | February 2010 | M | + | 0 | 1,4,5,12:i:1,2 | crop | U277 | 0 | + crop |
| Eurasian Siskin | Styria (Weiz) | February 2010 | M | 0 | + | 1,4,5,12:i:1,2 | liver | U277 | + | ND |
| Eurasian Siskin | Styria (Rottenmann) | February 2010 | M | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Styria (Rottenmann) | February 2010 | M | + | + | ND | ND | ND | + | + crop/+ lung |
| Eurasian Siskin | Vorarlberg (Bürs) | March 2010 | M | 0 | + | 1,4,5,12:i:1,2 | liver | U277 | + | ND |
| Eurasian Siskin | Vorarlberg (Dornbirn) | March 2010 | M | 0 | + | 1,4,5,12:i:1,2 | liver | U277 | + | + lung |
| Eurasian Siskin | Vorarlberg (Hohenems) | March 2010 | M | + | 0 | 1,4,5,12:i:1,2 | crop | U277 | + | + crop/+ lung |
| Brambling | Vorarlberg (Dornbirn) | March 2010 | M | 0 | 0 | 1,4,5,12:i:1,2 | liver | U277 | + | ND |
| Chaffinch | Vorarlberg (Dornbirn) | March 2010 | M | 0 | 0 | 1,4,5,12:i:1,2 | liver | U277 | + | ND |
| Bullfinch | Salzburg (Saalfelden) | March 2010 | F | 0 | 0 | 1,4,5,12:i:1,2 | heart, liver, eye, kidney, eyelid | U277 | + | + lung |
| Goldfinch | Vorarlberg (St. Gallenkirch) | February 2012 | M | + | + | 1,4,5,12:i:1,2 | crop | U277 | 0 | + crop |
| Eurasian Siskin | Vorarlberg (St. Gallenkirch) | February 2012 | M | + | + | 1,4,5,12:i:1,2 | crop | U277 | 0 | + crop |
| Eurasian Siskin | Vorarlberg (St. Gallenkirch) | February 2012 | M | + | + | 1,4,5,12:i:1,2 | crop | U277 | 0 | + crop |

[a] GIT = gastrointestinal tract; IHC = immunohistochemistry; F = female; M = male; + = positive; 0 = no changes; ND = not done.

[b] Bacteria in inner organs diagnosed by histopathology.


FIGURE 1. Map of Austria, showing the locations of 24 proven cases of salmonellosis in Fringillidae during the winter months in 2010 (15 Eurasian Siskins [*Carduelis spinus*], four Eurasian Bullfinches [*Pyrrhula pyrhulla*], one Brambling [*Fringilla montifringilla*], and one Chaffinch [*Fringilla coelebs*]) and 2012 (two Eurasian Siskins, one European Goldfinch [*Carduelis carduelis*]). Each circle represents one site at which a mortality was reported; filled circles indicate the year 2010, and the open circles indicate the year 2012.

subsequently subcultured onto XLD agar (Difco) incubated aerobically at 37 C for 24–48 h. Presumed *Salmonella* colonies on XLD agar (black or black-centered, $H_2S$-positive; pinkish-yellow, $H_2S$-negative) were confirmed by biochemical identification using API 20E (bioMérieux Austria GmbH., Wien, Austria). In all 14 cases, presumptive *Salmonella* isolates were submitted for serotyping and phage typing to the National Reference Centre for Salmonella at the AGES (Austrian Agency for Health and Food Safety) in Graz, Austria.

## RESULTS

In 2010, the first cases were noted in January, peaked during February, and ended at the end of March. A similar, slightly smaller outbreak was observed in February 2012. Both outbreaks were invariably associated with *Salmonella* Typhimurium and particularly occurred in the region surrounding artificial feeding sites. Deaths were observed in several areas quite distant from each other and located in three federal states of Austria (Styria, Salzburg, and Vorarlberg; Fig. 1). Eyewitnesses reported that, in some cases, hundreds of small songbirds were lying dead on the ground. Bird watchers described moribund birds that were unable to fly away, with fluffed and ruffled plumage and some with beaks clogged with feed. There were no signs of diarrhea. During January to March 2010 and February 2012, a total of 34 songbird carcasses were examined. Most ($n$=27) were Eurasian Siskins (*Carduelis spinus*); other species submitted were four Eurasian Bullfinches (*Pyrrhula pyrrhula*), one Brambling (*Fringilla montifringilla*), one Common Chaffinch (*Fringilla coelebs*), and one European Goldfinch (*Carduelis carduelis*; Table 1). They were all identified as adults. Two birds were female, and 32 were male. All investigated carcasses were in poor nutritional status with marked atrophy of the pectoral muscles and an absence of fat reserves. The average values for body weight were 9.8 g for the siskins and 22 g for the bullfinches. In 10 birds, pathologic examination was considerably impaired because of poor carcass preservation; therefore, only a macroscopic evaluation was performed. In those cases, poor nutritional status was the most striking finding. In 18 of the 24 examined birds (75%), the crop, esophagus, or both had multifocal to coalescing, yellowish nodules or plaques, with an average size of 1–3 mm, some of which obstructed the lumen (Fig. 2a and Table 1). Histologically, a severe, acute, frequently transmural, fibrino-purulent to necrotizing pharyngitis, ingluviitis, or esophagitis, with the presence of numerous intralesional bacterial colonies, was found (Fig. 2b). In eight cases (33%) with macroscopically recogniz-

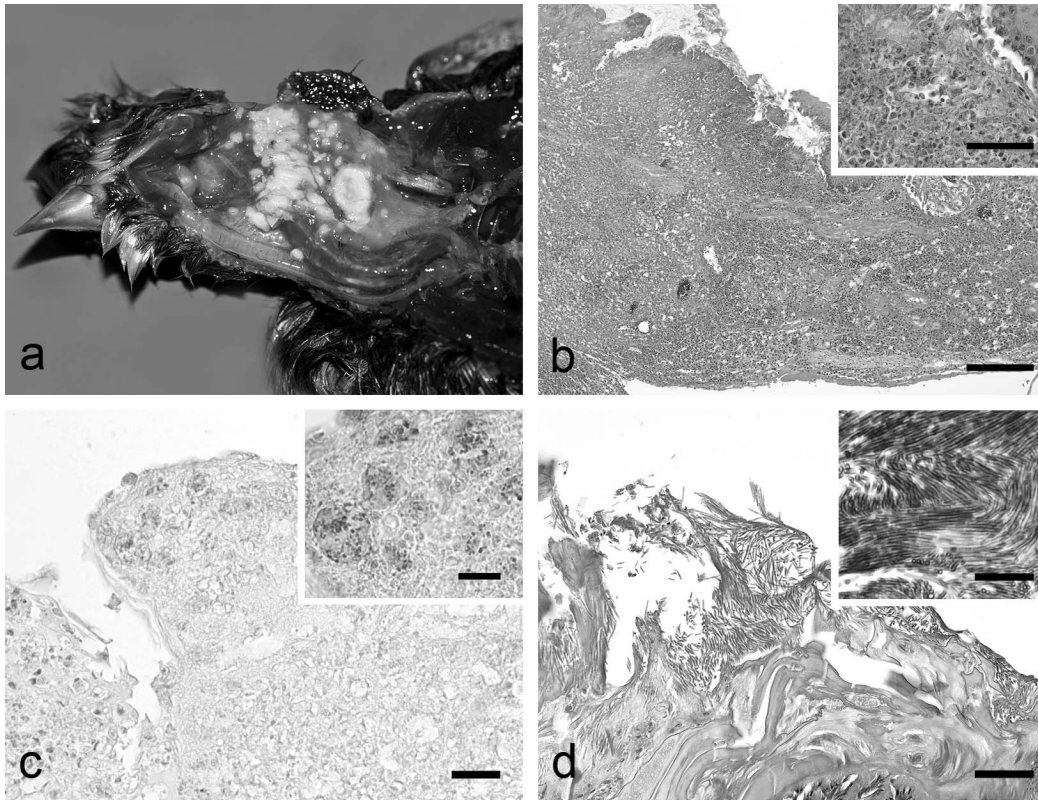


FIGURE 2. (a) Eurasian Siskin (*Carduelis spinus*) with extensive multifocal to coalescing nodules in the crop/esophagus obstructing the lumen. (b) Crop wall of the same bird with severe transmural purulent inflammation and mucosal ulceration. H&E. Bar=160 μm. *Salmonella* Typhimurium was isolated from these lesions. Inset shows necrosis, fibrin formation, and infiltration of (partially degenerated) neutrophils. H&E. Bar=40 μm. (c) In this bird, the presence *Salmonella* is immunohistochemically demonstrated as a brown reaction product in the crop mucosa. Bar=20 μm. Inset shows higher magnification of the labeled bacteria. Bar=10 μm. (d) In the same case, there is also histologic evidence of structures that are in morphologic accordance with *Macrorhabdus ornithogaster* on the surface of the gizzard. H&E. Bar=50 μm. Inset shows numerous, densely packed fungal elements specifically stained with the Grocott's methenamine silver method. Bar=25 μm.

able crop changes, *Salmonella* was isolated from these lesions in the upper digestive tract, and in five cases (21%) without typical crop or esophageal lesions, *Salmonella* was isolated from the liver. In one animal, also without crop or esophageal changes, *Salmonella* was cultivated from liver, heart, kidney, eye, and eyelid. All *Salmonella* isolates were determined to belong to the serovar Typhimurium with the antigenic formula 1,4,5,12:i:1,2 and phage type U277. In 17 birds (71%; 11 birds with classical crop lesions; six animals with proven *Salmonella* infection but without crop lesions), there was histologic evidence of septicemia, with multifocal bacterial colonies in the liver and brain, sometimes with accompanying purulent inflammation. Immunohistochemical examination identified *Salmonella* in the crops, lungs, or both of 19 birds (79%; Table 1). Numerous bacteria were found intralesionally in the transmural fibrino-purulent to necrotizing inflammation of the crop and esophageal mucosa (Fig. 2c). In blood vessels or capillaries of 11 lungs (46%), only small amounts of positive signals were detectable.

Additionally, in 17 birds (71%), we noted the presence of elements morphologically resembling *Macrorhabdus ornithogaster*, at the transition from proventriculus to gizzard,

without an inflammatory reaction (Fig. 2d and Table 1). The presence of these *M. ornithogaster*–like structures was confirmed by Grocott's methenamine silver stain.

No other lesions were found. In particular, no evidence of mycobacterial infection was detected by histologic examination. In all birds with ingluviitis, esophagitis, or both, infection with *Trichomonas* spp. was excluded by PCR.

## DISCUSSION

Passerine birds in general are very susceptible to *Salmonella* infection, which is, therefore, considered an important wild-bird disease (Hall and Saito 2008). The most common serovar associated with wild-bird salmonellosis is *Salmonella* Typhimurium, which has also been detected in healthy, free-ranging passerine birds and some gull species (Williams et al. 1976; Tizard et al. 1979; Quessy and Messier 1992; Refsum et al. 2002). However, the overall prevalence of *Salmonella* in healthy wild birds appears to be very low, especially in birds that live far from farms, sewage plants, or feed yards (Tizard 2004). Our study showed that *Salmonella* Typhimurium 1,4,5,12:i:1,2, phage type U277, was responsible for the mass mortality of songbirds in Austria during the winter months in 2010 and 2012. Although such events have been frequently reported in many parts of the world, outbreaks of the described size and intensity had not previously been described in Austria. In contrast to some other reports (Refsum et al. 2002; Lawson et al. 2010), only a single phage type (U277) was detected. Interestingly, the affected birds were found in three federal states and in different years, making a single large infection cluster unlikely. Most of the infected birds showed severe fibrino-purulent inflammation in the pharyngeal, ingluvial, or esophageal mucosa. In addition, there was evidence of septicemia by histologic as well as bacteriologic and IHC examination in some birds. Septicemia was identified as the cause of death in some of the birds and starvation because of painful inflammation and mechanical blockage of the upper digestive tract in others. These findings are largely consistent with other reports of *Salmonella*-associated mortalities in passerine birds worldwide (Daoust et al. 2000; Refsum et al. 2003; Hernandez et al. 2012; Giovannini et al. 2013; Fukui et al. 2014). Giovannini et al. (2013) described a very similar *Salmonella*-triggered mass mortality of Eurasian Siskins in the winter of 2010 in neighboring Switzerland. The birds showed identical crop and esophageal lesions with necrotizing esophagitis, ingluviitis, or both. Similarly, serotyping of *Salmonella* yielded an almost identical result. The only difference was agglutinations with antisera against O1 and O5 were positive in our cases (compared with negative agglutinations against O1 and O5 in Switzerland). Nonetheless, it is very likely that Giovannini et al. (2013) and the present study refer to the same larger, transboundary infection event in Central Europe. A morphologically indistinguishable inflammation of the upper digestive tract can also be induced by infection with other pathogens, such as *Trichomonas gallinae*, which is a very frequent cause of death in the Greenfinch (*Chloris chloris*; Lawson et al. 2012). Thus, a method for localizing the responsible pathogens within histologic slides is a useful diagnostic tool. The IHC supported the etiologic diagnosis in cases in which bacteriologic tests were not performed. As seen previously (Daoust et al. 2000; Refsum et al. 2003; Giovannini et al. 2013), the birds of the present study presented in a poor nutritional status with marked atrophy of the pectoral muscles and absence of fat reserves. Because infection with *Salmonella* induces an acute inflammation, it is very unlikely that the short time in which the birds were unable to feed led to those dramatic losses of body weight. It is more probable that the poor state of nutrition and associated weakening of the birds resulted in increased susceptibility to this infection (Tizard 2004). Although the birds were found dead near feeding sites, suggesting unrestricted access to feed, at least immediately before death, the poor nutritional state indicates a longer period of starvation. A

possible explanation for this could be specific weather conditions in the respective period. The mortality events occurred during a harsh winter with large snow masses and very low temperatures. According to meteorologic data (ZAMG 2010), 2009–10 was a very cold winter, especially in December and January. In addition, snowfall was 130–190% above the average. These weather conditions, with their associated, reduced natural-feed supplies, exposed the wild birds to an enormously stressful situation, possibly leading to emaciation and immunosuppression. This may also have caused the affected Fringillidae species to leave their natural habitats and to visit artificial feeding sites. It is a well-known problem of wildlife feeding that there is a high degree of fecal contamination at such feeding sites; thus, the infection pressure is increased considerably (Refsum et al. 2003; Lawson et al. 2010). Contamination of grains with bacteria is particularly unavoidable, especially when the feed is scattered on the ground where the birds frequently defecate among it. Unfortunately, most feeding places are not designed to prevent fecal contamination.

The dominance of siskins that we noted has also been observed in other *Salmonella*-induced mortality events (Refsum et al. 2003; Giovannini et al. 2013). Siskins may be especially vulnerable because of their feeding habits. According to Daoust et al. (2000), finches tend to feed on the ground and on feeders for prolonged periods, which increases the risk of the birds dropping feces onto food or being exposed to contaminated feed. Refsum et al. (2003) also pointed out that these sociable birds prefer feeding on the ground in groups and can, therefore, be easily infected via contaminated seeds. In addition, the way the grains are eaten has an influence on the risk of infection because, for example, tits (*Paridae*) pick the grains and hold them between their feet. Finches, including siskins, on the other hand, may hold several seeds in their beaks and peel them one by one (Refsum et al. 2003). In contrast, other bird species, such as Black-capped Chickadees (*Poecile atricapillus*), only visit the bird feeder for short periods to grab a single seed, leaving very quickly (Daoust et al. 2000). Another reason for the extraordinarily high number of dead Eurasian Siskins could simply be the presence of an above-average number of birds of this species in Austria in the mentioned winter seasons. The Eurasian Siskin is a partial migrant, whose northern populations move to Central Europe or even further south in autumn. However, those winter migrations fluctuate strongly from year to year. An important driver of migration seems to be the food supply. Siskins are inhabitants of mountain forests, with spruce or other coniferous trees, as well as mixed forests up to altitudes around 1,800 m. A high concentration of Eurasian Siskins has been seen to coincide with local spruce masting years (Bezzel 1992). In 2009, in northeastern Switzerland and in Austria, the seed production of spruces was significantly increased (Burri et al. 2016). This increased supply of conifer seeds, especially those of the spruce, is a decisive prerequisite for the development of local concentrations of siskins.

Intriguingly, several birds were also severely affected by structures morphologically resembling *Macrorhabdus ornithogaster*. However, no signs of inflammatory response were found (Legler et al. 2015). Finding individual seeds in the beak cavity or crop could indicate choking and vomiting or problems with swallowing or impairment of the normal transport of feed through the esophagus because of the inflammation caused by *Salmonella*. Additionally, in most cases, the digestive tract of the birds contained only a few grains of feed, and three of them showed signs of intestinal hemorrhages, which suggests at least 24 h of abstinence from food (Dorrestein and Kummerfeld 2014). Such findings are often seen in *Macrorhabdus* infections. *Macrorhabdus ornithogaster* has previously been found in wild finch populations (Pennycott et al. 1998) and is associated with emaciation and an abnormal intestinal bacterial flora (Legler et al. 2015). In our cases, it was not possible to determine whether the infection with *M. ornithogaster*–like structures was a cause or consequence of

the emaciation. The presence of large quantities of these fungi is highly unphysiologic, even in absence of proventricular inflammatory lesions. Assuming that yeast colonization to such a large extent takes some time, we may speculate that this condition might have been a predisposing factor for the *Salmonella* infection.

The very high percentage (94%) of male animals in the present study is exceptional. A similar, uneven sex distribution has been observed by Bezzel (1992) based on catches of living birds on control spots (70 males vs. 6 females). A possible explanation for this phenomenon could lie in the earlier mentioned abundant feed supply in the respective years: this could have led to dense and almost colony-like breeding attempts at the beginning of winter, which, in consequence, decreased mobility of females and significantly reduced their visits to feeding places.

A high bacterial load at the feeding sites carries a high risk of infection for other animal species, such as cats. In Switzerland, frequent cases of *Salmonella* infections with fever and gastrointestinal symptoms were reported in cats that had apparently eaten infected birds (Giovannini et al. 2013). Cases of *Salmonella* infection in humans after direct or indirect contact with wild birds have also been repeatedly described (Macdonald and Brown 1974; Penfold et al. 1979; Kapperud et al. 1998; Tauni and Osterlund 2000; Alley et al. 2002; Thornley et al. 2003; Nesse et al. 2005; Tsiodras et al. 2008; Hauser et al. 2009). However, during our investigations, we did not become aware of any increased *Salmonella* infections in cats or humans in Austria.

In conclusion, this study proved that *Salmonella* Typhimurium was responsible for the frequent deaths of wild songbirds in Austria in the winter months of 2010 and 2012. In all cases, a single variant of *Salmonella* Typhimurium (1,4,5,12:i:1,2; U277) was involved.

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