Risk of Salmonella-introduction at dairy farms caused by Dutch wildlife

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Abstract

To maintain the high quality of their dairy products, Dutch farmers are required to produce *Salmonella*-free milk. Wildlife introduction is one of several ways to introduce a *Salmonella*-infection on a dairy farm. Because of the lack of *Salmonella*-prevalence studies in Dutch wildlife, the present study observes *Salmonella* prevalence in Salland, the Netherlands.

121 animals were collected from and near dairy farms in Salland, from which two *Salmonella*-positive samples were obtained from two rats. The study also included 25 rats from two different dumpsites near the region of Salland, which were all *Salmonella*-free.

While the present study has a sufficient number of samples per km2, the sample pool is less representative of real-life wildlife diversity. To estimate the risk wildlife presents for dairy farms, different animal groups have been investigated. For dairy farms in Salland, the biggest risk for *Salmonella*-introductions are presented by carrion birds and gulls, followed by large flocks of small garden birds. A special case can be made for rats and mice, that rarely introduce *Salmonella* themselves. Instead, they could maintain the on-farm infection as reservoirs, similar to cats and dogs. Other species, like raptors, carnivores, deer, and wild boars are considered low-risk. Finally, the risk presented by moles, hares, and wild rabbits is uncertain.

Keywords: *Salmonella, Salmonella* prevalence, wildlife, dairy farms

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Introduction

Dutch dairy product manufacturers are known for good product quality. To maintain said quality, these companies only accept milk from dairy farmers that meet their requirements. The requirements vary from the freezing point of milk, to cell count, to being free from certain pathogens. An example of such a pathogen is *Salmonella*, a bacterium known to be transmissible between humans and animals – also referred to as zoonosis.

Of all human *Salmonella*-cases in the Netherlands 3,3% are suspected to have originated from cattle in 2015. The report did not discriminate between milk and beef products (Uiterwijk et al., 2007). These low values suggest there are only a few *Salmonella*-positive cattle herds in the Netherlands. In comparison to other European countries, in the Netherlands single-case incidents do not have to be reported to the Dutch authorities (Graveland et al., 2013; Uiterwijk et al., 2017). This could explain the low *Salmonella* case rate compared to other countries.

Of all human *Salmonella*-cases, 70% are caused by *Salmonella enteritidis* and *Salmonella typhimurium*. This value has been stable, though even *Salmonella* spp. pathogenicity can change. In the other 30% of the cases a new pattern has been observed in both the Netherlands and other EU State members. This pattern is caused by a new 'emerging'-type of *S. typhimurium*, which can be considered a third subspecies of *Salmonellea* that is found in the majority of human infection cases.

The European Regulation (EC) No 2160/2003 states

… to ensure that proper and effective measures are taken to detect and to control *Salmonella* and other zoonotic agents at all relevant stages of production, processing and distribution, particularly at the level of primary production, including in feed, in order to reduce their prevalence and the risk they pose to public health. (Chapter 1, article 1).

The Dutch government has delegated the task of monitoring and controlling these regulations to the relevant sectors. In case of the dairy sector, all relevant parties are joined in the Dutch Dairy Organisation (NZO). Although milk is considered less likely to cause human-salmonellosis in the Netherlands, new measures taken by the NZO also improve the secure status of their product quality.

The NZO require all delivering dairy farms to participate in the *Salmonella*-Unsuspected Program. This means every farm's milk is tested for antibodies and bacteria of *S. enteritidis* and *S. typhimurium* three times a year (GD, 2014a). When unfavourable outcomes are tested four times in a row, measures are taken on the particular farm (GD, 2014b). This includes culling carriers, improving work routes and management to prevent further spread on the farm, and tracking down the origin of the *Salmonella*-introduction. A *Salmonella*-introduction can be caused by purchased cattle, purchased manure, veterinarians and other visitors, lending machinery and wildlife. This study aims to gain more insight into how wildlife could cause a *Salmonella*-introduction at dairy farms.

Currently, consumers and customers encourage and reward dairy farms that allow grazing. This grazing management increases the chances of direct and indirect contact between cattle and wildlife. Moreover, Dutch cattle stables tend to have an open structure to improve the housing climate, which allows easier entry of wildlife. According to Jorritsma

and Hofste (2011) pests and birds are risk factors for prolonged *Salmonella*-antigen positive Dutch dairy farms. Also, Gortázar, Ferroglio, Höfle and Frölich, Vincente (2007) concluded that an overgrown wildlife population and open-air farming are risk factors for spill-over between livestock and humans.

Recently wild mice in and around the city of Utrecht were sampled for pathogens. Burt, Meijer, Burggraaff, Kamerich and Harmanus (2018) found no *Salmonella*-positive samples. In another study, rodents and insectivores were sampled at nine organic pig farms and one organic poultry farm in the Netherlands (Meerburg, Jacobs-Reitsma, Wagenaar & Kijlstra, 2006). Of the 282 samples, one house mouse (*Mus musculus*) outside the farm tested *Salmonella*-positive. Also, the GD Animal Health examined different factors that could influence the *Salmonella* status of a dairy farm. Commissioned by NZO, the GD Animal Health investigated the relationship between oversummering geese and prolonged *Salmonella*-antigen positive farms. On each farm, 100 fresh geese faeces were collected on meadows on which cattle and geese graze. The prevalence of *Salmonella* in the geese faeces were calculated at 0,04% (Weber & Heuvelink, 2013).

Similarly, in other European countries the *Salmonella*-prevalence in wild birds has been observed. In Sweden the prevalence in migratory birds was determined by Hermandez, Bonnedahl, Waldenström, Palmgren and Olsen (2003). Out of the 2377 samples taken in autumn, winter and spring, one *Salmonella*-positive sample was cultured. In another study in Sweden the influence of black-headed gulls (*Larus ridibundus*) on *Salmonella*-epidemiology was studied (Palmgren et al., 2006). Of the 1047 samples 28 (2,7%) were *Salmonella*-positive. In Norway, Refsum, Vikøren, Handeland, Kapperud and Holstad (2003) sampled dead birds found in gardens to determine the prevalence of *Salmonella,* which was 69% (123/179).

A similar study in England and Whales by Lawson et al. (2010) had a prevalence of 22% (157/698). In Croatia 8 of the 107 wild, living birds (7,5%) tested positive for *Salmonella* (Vlahovic et al., 2004). In Spain of the 97 clinically healthy Griffon vultures (*Gyps fulvus*) 51 animals (52,6%) had a positive culture (Marin, Palomeque, Marco-Jimenez & Vega, 2014).

Equally important are the European studies which observed the *Salmonella*-prevalence in wildlife. In 1998-1999 Wahlstörm et al. (2003) asked Swedish hunters for faecal samples of Canadian geese, deer, hares, moose, gulls, and wild boars. Of the 696 samples only 4 gulls were *Salmonella* positive. Millán, Aduriz, Moreno, Juste and Barral (2004) found a prevalence of 7,8% in wildlife, consisting of 7 out of 82 birds, and 9 out of 123 mammals in Basque Country in Spain in 2001-2002. The positive samples were taken from raptors, carnivores and wild boars. Another study in Catalonia took samples from asymptomatic animals in the local Wildlife Rehabilitation Center (Molina-López, Vidal, Obón, Martín & Darwich, 2015). They observed a prevalence of 4,2% (11/263). The sample pool consisted of mammals, birds and reptiles. Skov et al. (2008) used a similar sample pool to Molina-López et al. (2015), but also included insects. Uniquely, Skov et al. (2008) first considered the *Salmonella* status of the sample locations prior to sampling. At *Salmonella*-negative locations the *Salmonella* prevalence was 0% in all species, while at positive locations a prevalence of 22,6% in insects, 5,2% in rodents, 6,5% in pets and 1,5% in birds was observed. Skov et al. found that, with a single exception, all *Salmonella*-types in wildlife were similar to the types of the closest *Salmonella*-positive farm.

There are also studies that have observed the *Salmonella*-prevalence in game meat. In those cases, samples are taken of the carcass, faeces or intestines. Especially the samples taken from the carcasses were more likely to be incongruent with results of aforementioned

studies. *Salmonella* prevalence could be lower within the carcass, because it is an unusual place for *Salmonella* to reside in in an asymptomatic animal. However, it could also be higher due to contamination during skinning (Paulsen, Smulders & Hilbert, 2012).

The present research has two aims. The first is to determine the *Salmonella* prevalence in Dutch wildlife. The secondary aim is to estimate the risk of *Salmonella*-introduction at Dutch dairy farms by wildlife.

Materials and Methods

Sample collection

Based on previous studies, this study assumed a possible *Salmonella*-prevalence of 5% in Dutch wildlife (Millán et al., 2004; Molina-López et al., 2015; Skov et al., 2008; Wahlstörm et al., 2008). According to the following formula, this study needed a minimal of 73 samples at a confidence of 95% (Z=1,96), an expected prevalence of 5% (P=0,05) and a precision of 5% (d=0,05) (Naing, Winn & Rusli, 2006).

$$n = \frac{Z^2 P(1-P)}{d^2}$$

A part-time hunter, a professional pest catcher, and a student collected the samples in Salland near dairy farms in the period of January 14th 2017 to March 4th 2017. The animal bodies, after being caught and killed, found dead, or shot dead were stored at temperatures ranging between -6°C and +3°C. Within 24 hours after death the faecal samples and/or intestines were taken from the bodies. Those samples were stored at 2°C to 4°C. To avoid (cross)contamination, the sampling was performed using disposable plastic bags. In the lab the faeces and the intestines' contents were mixed before the isolation procedure started. To

verify the *Salmonella* status of particular dairy herds, twelve pooled faecal samples were used. Each sample pool consisted of five samples of fresh collected material from different cows.

Isolation procedure

Salmonella was isolated according to the methods of ISO-standards with a small adjustment. One to twenty-five grams of faeces were places in a Buffered Pepton Water (BPW), with a ratio of 1-gram faeces to 9 mL BWP, and vortexed or shaken for 90 sec. The BPW was incubated at 37°C for 24 hours. A total volume of 0,1mL of sample-BPW was taken into a Rappaport-Vassilliadis *Salmonella* medium (RVS), which was incubated at 42°C for 24 hours. Another sample of 1,0mL of sample-BPW was put into a Muller-Kauffman Tetrathionate Novobiocin medium (MKTN), which was incubated at 37°C for 24 hours. Afterwards both samples of RVS and MKTN were streaked for isolation on a Briljant Green Agar (BGA) and a Xylose Lysine Deoxycholate (XLD) plates. Also, a *Salmonella*-positive control was made for each plate type. Those were incubated at 37°C for 24 hours.

The possible *Salmonella* positive colonies were transferred to a Trypticase Soy Agar (TSA) plate and incubated at 37°C for 24 hours. The colonies of the TSA plates could be used for the *'Korte Bonterij'*, that consisted of a Triple Sugar Iron agar (TSI), a Urea Broth base (U) and a Lysine Decorboxylase test (LDC). Those were also incubated at 37°C for 24 hours. The positive samples were tested with an agglutinate test of a *Salmonella* Polyvalente O A-S rabbit serum.

Results

The initial aim of the present study was to collect 73 animals/droppings in seven weeks. However, a total of 146 samples were collected. All samples meet the requirements of originating from or near a dairy farm in Salland, excluding 25 rats. Those 25 rats originated from two dumpsites (ROVA®) near the region of Salland. The distribution of animal categories and species are illustrated in figure 1.
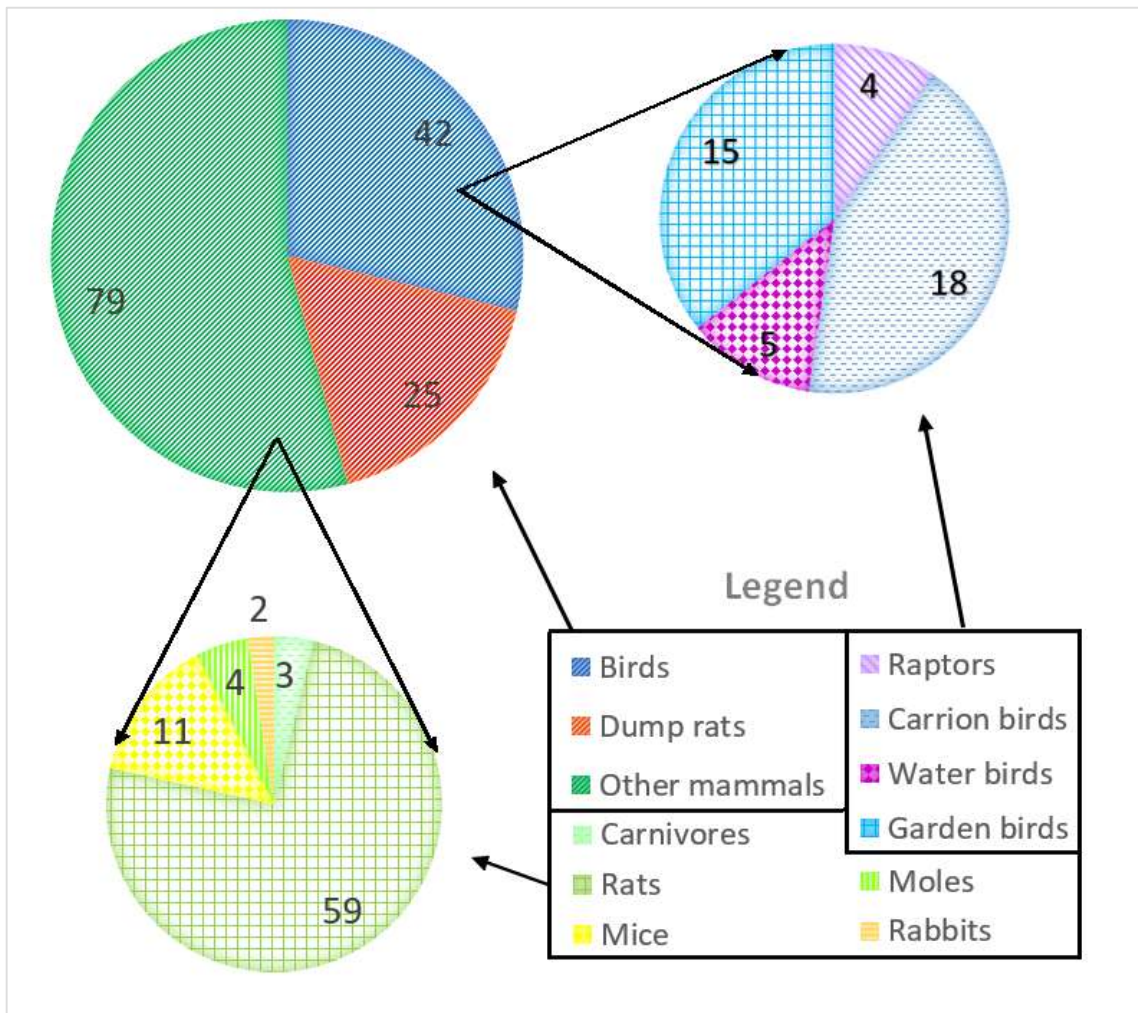

Figure 1 Distribution of all samples in the current study

In figure 1 a distinction is made between birds, mammals, and rats from the dumpsites. The mammals consisted of 59 brown rats (*Rattus norvegicus*), 11 house mice (*Mus musculus*), 4 European moles (*Talpa europaea*), 2 European rabbits (*Oryctolagus*

cuniculus), 2 red foxes (*Vulpes vulpes*) and 1 wildcat (*Felis silvestris*). The 25 rats from the

dumpsites were also brown rats. The birds included 12 common starlings (*Sturnus vulgaris*),

10 carrion crows (*Corvus corone*), 8 western jackdaws (*Coloeus monedula*), 2 common wood

pigeons (*Columba palumbus*), 2 barn owls (*Tyto alba*), 2 buzzards (*Buteo buteo*), 2 mallards

(*Anas platyrhynchos*), 1 greyleg goose (*Anser anser*), 1 Egyptian goose (*Alopochen

aegyptiaca*), and 1 black-headed gull (*Chroicocephalus ridibandus*). One unidentified guano

sample was also analysed. The samples taken from the barn owls were fresh guano, and an

owl ball. The unidentified guano sample was taken from inside the barn.

Discussion

Sample pool size

The first aspect of this study is to decide whether the used sample pool is

representative of the wildlife population in Salland or the Netherlands. Maas et al. (2016)

noted that in the Netherlands there is no national wildlife counting. Additionally, at the

present time there are no known studies that observed Dutch wildlife diversity, in either

numbers or prevalence. Thusly, the current study will be compared to similar *Salmonella*-

observing studies in Europe. They are summarized in table 1.

Table 1 Studies used to compare current study stated with number of samples, mammals, bird, sample location and sample per km2.

Author	Total samples	Mammals	Birds	Region, land	Sample area (km2)	Sample /km2
Current study	146	102 (70%)	44 (30%)	Salland, the Netherlands	855	0.171
Millán et al. 2004	205	123 (60%)	82 (40%)	Basque Country, Spain	7.235	0,028
Molina-López et al. 2015	263	50 (19%)	169 (64%)	Catalonia, Spain	32.108	0,008
Skov et al. 2008	2933	225 (10%)	2567 (88%)	Denmark	16,5	117,8
Wahlstörm et al. 2003	696	585 (84%)	111 (16%)	Sweden	855.215	0,001

The first thing that stands out in table 1 is the different percentages of mammals and birds used in the studies. The high percentage of mammals in the current study can be explained by the use of a professional pest catcher, that caught mainly rats, mice and moles. The other animals were shot by part-time hunter. Millán et al. (2004) also used hunters, who shot mainly even-toed ungulates and red foxes. The other animals were found dead, mainly road-killed. Contrarily, Molina-López et al. (2015) collected the samples of living asymptomatic animals in a wildlife rehabilitation center. The center also contained reptiles, which represent 17% of the sample pool. Likewise, Skov et al.(2008) took samples of living animals. Traps were used to catch rats and mice on farms and other locations, while birds were caught by professional ringers on said locations as well as 100m and 500m away from there. The remaining 2% of the sample pool was made up by insects on location.

All named studies – including the present study – used a partially randomized sample pool by the very nature of the collection method. The use of professionals to collect samples could be seen as a factor causing a bias. Their efficiency in collecting samples influenced the randomized sample pool, which, if undisturbed, should be comparable to the real wildlife population. Using professionals to collect all samples should reduce the collection bias. Examples are the current study and Skov et al. (2008). Wahlstörm et al. (2003) used professionals exclusively for their sample collection, but the sample pool size was predetermined. Wahlstörm et al. did not justify their sample pool numbers, though it can be said they are not a representation of the natural wildlife ratios.

The second thing that stands out in table 1 is the samples taken per km^2. Skov et al. (2008) has the highest number of samples per km^2, caused by the way he marked the sampled areas. They only selected 21 locations with a radius of 500m. The other studies did not use such a specific area. In the case of Molina-López et al. (2015) samples were taken in one

location – namely the wildlife rehabilitation center – though the animals may have originated from a larger area.

The sample pool of the current study is not representative of the real wildlife population, as a result of using a professional pest-catcher. In the future – to prevent this kind of bias – the working hours of the pest-catcher and hunters should evenly distributed. In the case of samples taken per km^2, the current study has a good number. Still, the number of samples taken from each species is too small to determine the correct *Salmonella*-prevalence for wildlife or each specie.

Prevalence

Wildlife

In the current study a *Salmonella* prevalence of 1,4% (2/146) was found. The findings of Millán et al. (2004) with 7,8% (16/205), Molina-López et al. (2015) with 5,0% (11/219), Skov et al. (2008) with 0,9% (27/2933) and Wahlstörm et al. (2003) 0,6% (4/696), indicate a lot of differentiation between *Salmonella* prevalence.

The current study and Wahlstörm et al. (2003) only found *Salmonella*-positive samples in one species, while the other three studies found in it in more animal species. So, it is not clear if all the wildlife has the same *Salmonella*-prevalence and same risk to introduce *Salmonella* at dairy farms, or only a couple species should be repelled from farms.

Therefore, the following sections will be discussing the potential *Salmonella*-prevalence in the Netherlands and the risk for Dutch diary farms of these animal groups: carrion birds, raptors, gulls, garden birds, waterfowl, rodents/pests, moles, hares and wild rabbits, carnivores, deer and wild boars.

Carrion birds

Carrion birds are known for their eating behaviour, which includes cadavers. This causes scavengers to have a higher risk of infection, including *Salmonella* (Tizard, 2004). In the case of Salland's dairy farms, the relevant birds are carrion crows (*Corvus corone*) and western jackdaws (*Coloeus monedula*). Those animals are found mostly near silage on farms, which may cause contamination of the feed.

In the current study no positive samples were found in the 18 birds. Contrarily, Millán et al. (2004) and Molina-López et al. (2015) found 20% (1/5) and 100% (2/2) prevalence respectively. However, the small sample pool size and the fact that these findings concerned vultures makes it less relevant to the Dutch situation.

Aside from those more general wildlife studies, there is also a number of studies that only observed carrion birds. Literak, Vanko, Dolejska, Čížek and Karpíšková (2007) found a *Salmonella*-prevalence of 2,5% (9/363) in the Czech rook (*Corvus frugilegus)* population. A comparative study between large corvidae-populations in Europe and North America found an overall-prevalence of 1,4% (39/2778) (Jonecko et al., 2015). No significant differences were observed between the two groups in the period of 2010-2013. In a study concerning an agriculture area similar to the Netherlands, *Salmonella* prevalence in Japanese crows was 8,9% (11/123) (Okumura et al., 2018). Notable were the cultured serotypes, of which the majority was uncommon in the Japanese livestock and citizenry. Faruq et al. (2016) found a 65% *Salmonella* prevalence in a study where 40 house crows (*Corvus splendens*) living near the Bengalese population.

Also, carrion birds are often included in studies that research the *Salmonella*-prevalence in the overall wild birds' populations. The following results were found for those scavengers: Lawson et al. (2010) found 0/7 positive *Salmonella* samples in *Corvidae* in the UK; Refsum et al. (2003) found 0/2 rooks in Norway; Pennycott, Park and Mather (2006)

sampled 2/19 dead or ill rooks in the UK; Vlahovic et al. (2004) found 2/13 rooks and 0/3 jackdaws in Croatia; Stenzel, Tykałowski, Mazur-Lech and Koncicki (2008) found 1/3 rooks and 2/8 jackdaws with positive antibodies to *Salmonella* in Poland; Handeland et al. (2002) found 1/6 hooded crows (*Corvus corone*) and 0/4 magpies (*Pica pica*) in Norway during a *Salmonella*-epidemy in hedgehogs.

There is a good possibility that Dutch carrion birds could have a similar prevalence as in Japan - namely 8,9% (Okumura et al., 2018). Firstly, this conclusion is based on the agriculture systems and population density – excluding mountains –, that are rather similar in Japan and the Netherlands. Secondly, although the number of samples in the overall bird studies is low, positive samples have been found in carrion birds. Thirdly, the studies by Literak et al. (2007) and Jonecko et al. (2015) were taken in areas with a lower human population density than in the Netherlands, which could explain the low values of 1,4% and 2,5% respectively. And fourthly, a *Salmonella*-prevalence of 65% that Farug et al. found is not applicable to the Dutch context, due to the difference in culture and public order regulations between Bangladesh and the Netherlands.

Raptors

Raptors are birds located at the top of the food chain. High *Salmonella* prevalence has been found for raptors (Tizard, 2004). In contrast to carrion birds, raptors are rarely found near stables with cattle or next to silages. Thus, they form a small risk for Dutch dairy farms. Nevertheless, the *Salmonella*-prevalence in this group of birds could provide an indication about the prevalence in their preys.

The current study contained 2 buzzards (*Buteo buteo*) and 2 barn owls (*Tyto alba*), from which no *Salmonella* was cultured. One of the owl samples was an owl pellet. This sample is included in the current faecal research, because owl pellets are made of remnants of

prey and are known to have enough bacteria to cause, for example, Salmonellosis-outbreaks (Smith, Anderson, Medus, Leano & Adams, 2005).

The comparable wildlife studies found a higher prevalence, Millán et al. (2004) 7,8% (4/51) and Molina-López et al. (2015) 6,2% (7/113). Because of the significantly bigger pool of raptors, those results seem more reliable than the current study. Moreover, in an earlier study of Molina-López et al. (2011), a prevalence of 12% (10/83) was found in diurnal owl species and 5,3% (2/38) in nocturnal owl species in Catalonia, Spain. In the general wild bird studies the following results were noted: Pennycott et al. (2006) found 1/5 sparrow hawks (*Accipiter nisus*) and 2/4 tawny owls (*Strix aluco*) that were *Salmonella*-positive in the UK; Vlahovic et al. (2004) found 2/22 raptors and 1/30 owls in Croatia; Stenzel et al. (2008) found 3/5 buzzards and 14/20 white storks (*Cicone ciconia*) that were sero-positive for antibodies.

To conclude, a *Salmonella*-prevalence of 5-20% is expected in Dutch raptors, based on the earlier stated studies. Although having a higher prevalence, they form a small risk for dairy forms. Those birds don't live often near the cows or their feed.

Gulls

Gulls are a special case, when living at the coast they behave as raptors but inland and near human civilization they behave as carrion birds. In the region of Salland they are often only seen in the summer, due to a lack of sea or big open waters. Big groups of gulls are often seen near dumpsites and after cultivating the ground. Some single individuals are seen near silages.

The current study did not culture *Salmonella* from the single gull sample, while Wahlstörm et al. (2003) obtained 4 positive samples of the 27, and Millán et al. (2004) 1/5 and Molina-López et al. (2015) 0/5.

Compared to raptors more studies about *Salmonella*-prevalence in gull populations have been conducted. Palmgren et al. (2006) found *Salmonella* in 2,7% of the 1047 black-headed gulls (*Larus ridibundus*) in Sweden. In Spain 17% (31/182) of the yellowleg gull-chicks (*Larus michahellis*) faecal cultures were *Salmonella*-positive (Ramos, Cerdà-Cuéllar, Ramírez, Jover & Ruiz, 2010). Čížek, Dolejská, Karpíšková, Dědičová and Literák (2007) observed different *Salmonella*-prevalence in black-headed gull-chicks in the Czech Republic throughout the decades. The period of '84-'86 had a prevalence of 12% (59/473), '91-'94 had 30% (100/331) and 2005 had 17% (48/291). In the same population as Čížek et al., Masarikova et al. (2016) observed 13% (37/284) *Salmonella*-positive chicks in 2012.

Similar studies outside of Europe have reported high *Salmonella* prevalence in gulls. In Australia Dolejská et al. (2015) sampled 13% (66/504) of the silver gull (*Larus novaehollandiae*) as *Salmonella*-positive. In Chili, near a fish-factory, Rodriguez et al. (2012) 60% (119/200) of kelp gulls (*Larus dominicanus*) and Franklin's gulls (*Leucophaeus pipixcan*) were faecal-positive. Moreover, Tizard (2004) stated that the increasing number of dumpsites, landfills and sewage outlets are important factors for the increasing *Salmonella*-prevalence in gulls the past decennia.

Notable is the number of studies that used chicks instead of adult gulls. In poultry it is known that the intestinal immune system, similar to the mammalian Peyer's patches, is noticeable in the second week after hatching. The patches will increase till 16 weeks of age (Beal, Powers, Davison & Smith, 2006). This developing immune system could also take place in gull-chicks, which might influence the *Salmonella*-prevalence in those studies. Ramos et al. (2010) also noted this point in their discussion. They noticed that *Salmonella*-infected gull-chicks had no changes in body condition compared to the negative gull-chicks. It confirmed their idea that gulls could also be merely non-affected carriers.

Based on the above, gulls really could be a big risk for dairy farms. The expected prevalence for the Dutch gulls living in the region of Salland is 10-20%. Firstly, thus conclusion is based on the fact that the gulls in Salland live close to humans and/or dumpsites. Secondly, the gull population of Cizek et al. and Masarikova et al. lived near great rivers and its villages. This compares to Sallands' landscape, in which the Ijssel-river is located. Thirdly, the waters in the Netherlands are more likely to be contaminated due to human interference. This is more similar to the Australian and Czech Republic environments than the waters in Sweden. And fourthly, although gulls are not often seen on the farms, they are seen near cultivated ground. They are seen especially often after fertilizing the ground with manure. These birds could be in contact with *Salmonella*-positive manure and spread to other agricultural lands. Depending on farms management, cows could be grazing in the same meadows in two weeks after fertilizing. It is also known that *Salmonella* could survive till 332 days in amended ground (Jacobsen & Beck, 2012).

Garden birds and waterfowl

For the purpose of this study, 'garden birds' refers to all small birds like starlings, sparrows, finches, etc., and pigeon species that are often seen in gardens. In the countryside, those birds are seen on farms. Some species nest in stables, other species search for food on the farms. Waterfowls in the Dutch context refers spefically to ducks and geese. Those two waterfowl species are more often seen on meadows. A remarkable similarity between those bird species is their appearance in large groups. While pigeons and ducks are more often seen in large groups in and near villages and cities, geese and garden birds, like starlings and sparrows, are seen in large numbers in the Dutch countryside. Tizard (2004) concluded in his review that large groups of birds have a higher risk of higher *Salmonella*-prevalence compared to individual or small groups, especially during mating, hatching and migration.

In the case of garden birds, the current study sampled 12 common starlings (*Sturnus vulgaris*) and 2 common wood pigeons (*Columba palumbus*), all *Salmonella*-free. In the similar wildlife study by Skov et al. (2008) 2549 small birds were sampled, including 40 starlings. The *Salmonella* prevalence of all those small birds was 0,8%, which included 1 positive common starling, 4/583 house sparrows (*Passer domesticus*) and 8/258 blackbirds (*Turdus merula*).

In the general wild bird studies large groups of small and/or garden birds were included. Lawson et al. (2010) found a prevalence of 26% (171/656) in diseased and dead birds in the UK. Especially the greenfinch (*Carduelis chloris*) with 61% (118/195), the chaffinch (*Fringilla coelebs*) 33% (6/18) and the house sparrow 28% (28/99) increased the prevalence. In the review of Tizard (2004) it was stated that a significantly different *Salmonella*-prevalence could be observed between samples taken from diseased or dead birds and living asymptomatic birds.

A good example for Tizard's statement is the study of Refsum et al. (2003). This study resulted in a *Salmonella*-prevalence of 69% (123/179) in dead small garden birds. In the same study 1.990 clinical healthy birds were cloacally swabbed, which resulted in a prevalence of 2%. Among the sample-pool of living birds the *Salmonella*-positive species were 8/281 Bullfinches (*Pyrrhula pyrrhula*), 2/390 Eurasian greenfinches (*Carduelis chloris*), 12/348 common redpoll (*Carduelis flammea*), 14/159 Eurasian siskins (*Carduelis spinus*), 2/25 house sparrows and 1/8 Bohemian waxwing (*Bombycilla garrulus*). The highest number of infected carcasses were found in the bullfinch (49/64), common redpoll (26/31) and Eurasian siskin (33/40). Also, Pennycott et al. (2006) sampled dead birds, and concluded a *Salmonella*-prevalence of 54% (196/361) in songbirds.

In the case of pigeons Vlahovic et al. (2004) found 2/14 *Salmonella*-positive pigeons and Pennycott et al. found (2006) 5/53. On the contrary, Molina-López et al. (2015), Skov et

al. (2008) and Lillehaug et al. (2005) did not find *Salmonella* in their 10, 11 and 100 samples respectively.

For waterfowls, the current study took samples from 2 ducks (*Anas platyrhnchos*) and 2 geese (1 greyleg goose, *Anser anser*; 1 Egyptian goose, *Alopochen aegyptiaca*), which were all negative. An important study for this group of birds was conducted by Weber and Heuvelink (2013), who found a prevalence of 0,04% in Dutch geese during the summer. Also, Vlahovic et al. (2004) – with 4 swans – and Molina-López et al. (2015) –with 17 waterfowls – found no positive samples. In a large study in Norway, Lillehaug et al. (2005) found 1 *Salmonella*-positive goose faeces in a pool of 219 geese and 5 ducks, so a prevalence of 0,5%.

In conclusion, individual and small groups of small garden birds are suspected to have a *Salmonella*-prevalence of 0,5-2,0%. This belief is based on the values found by Refsum et al. (2003) in living birds and Skov et al. (2008). In the case of large groups of these birds, prevalence could increase to over 50%. As mentioned earlier, there is a higher risk of higher *Salmonella*-prevalence in larger groups of birds (Tizard, 2004). This is caused by the difference in immune status of all the birds, which could increase the infection pressure in the entire group. In Refsum et al. (2003) and Lawson et al. (2010) it is seen that diseased and/or dead birds have a higher prevalence of *Salmonella*.

So, the biggest risk for diary farms are large groups of small garden birds, even if they only stay around silages or inside the stables for a short while. Individuals, small groups, and pigeons would be considered as a low-risk factor for potential *Salmonella*-introduction. The case is similar for geese and ducks. They should also be valued as a low-risk factor for potential *Salmonella*-introductions.

Rodents

At farms mice and rats are often seen nesting in stables or near silages. Those animals are seen as pests, because they damage electronics, take from animal feed, and are thought to carry a lot of pathogens. Especially at dairy farms, mice and rats can easily attain their feed and live next to it. So, there is a higher risk of feed-contamination by their faecal droppings.

With those thoughts, Meerburg et al. (2006) sampled wild small mammals inside and near organic farms in the Netherlands. They found 1 positive mouse in the pool of 274 mice and 8 rats. Also, Burt et al. (2018) researched the prevalence of different pathogens in mice in and around the city of Utrecht, the Netherlands. From the intestines of the 51 mice, no *Salmonella* spp. were cultured.

In the current study 2 of the 59 (3,4%) brown rats (*Rattus norvegicus*) in and near dairy farms were *Salmonella*-positive. This study also included 25 rats from two different garbage dumps, all *Salmonella*-free. Those animals were caught or shot by a professional pest catcher. This person also delivered 11 wood mice (*Apodemus sylvaticus*), which were all free from *Salmonella*. Similar to the current study, Skov et al. (2008) also caught mice and rats. At the *Salmonella*-unsuspected locations the prevalence was 0% (0/68), while the suspected locations had a prevalence of 5,2% (7/135).

Equally important is the study of Backhans et al. (2013) in which the *Salmonella* prevalence in brown rats and mice was observed in Swedish pig- and poultry farms and non-farm locations. Of the 56 rats and 129 mice, only 1 mouse carried faecal *Salmonella*. Distinctly different are the rat samples of Hilton, Willis and Hickie (2002) that live in urban regions in the West Midlands of the UK. In this study 5/50 rectum swabs were positive, as well as 8 of the 100 faecal droppings. This corresponds with the literature review of Paulsen et al. (2012) that found that wildlife in closer contact to humans and/or garbage has higher

Salmonella-prevalence. However, in a study on rats in Vancouver Canada, Himsworth et al. (2015) found a prevalence of only 0,5% (3/633).

The *Salmonella*-prevalence of rats and mice on dairy farms could be influenced by the *Salmonella*-status of the farm itself. When the farms are unsuspected the prevalence should be around 0-0,5%, while *Salmonella* prevalence of rats and mice on suspected locations could increase to 10%. This is based on the studies of Skov et al., Burt et al., Meerburg et al. and Himsworth et al. that suggested that rats and mice in wild nature often do not carry *Salmonella*, except near *Salmonella*-hotspots.

Rats and mice are low risk for introducing a *Salmonella*-infection on dairy farms, except when neighbouring farms are already *Salmonella*-positive. Instead, these pests increase the risk for farms to become long-term antigen-positive, as they are the in-farm reservoir for *Salmonella*.

Moles

The moles, caught by the pest catcher, are being treated as pests in the same way by farmers in Salland, as they are in most European countries (Du Bois, 2013). Moles live relatively close to the meadows, from which feed is obtained throughout the year. Additionally, those grounds are being cultivated with manure. The manure could be *Salmonella*-contaminated from the ground-owning farm itself, by colleagues' farms, or by loaning machinery. Only one of the earlier *Salmonella*-prevalence studies in wildlife, Jones and Twigg (1976), noted 7 *Salmonella*-free samples of moles. A similar result was seen in the current study with 4 moles. In other studies, moles or *Salmonella* were not included in their methods.

Du Bois (2013) noted in her literature study that an increase of mole activity is seen in spring and autumn. An increase of mole activity could also increase the possible indirect contact between moles and cattle. Different reasons are supplied for the increase of their

activity. Firstly, during spring and autumn the shallower ground contains more moisture, which is more favourable for moles. Secondly, the mating behaviour in the spring might increase mole-activity. Thirdly, during summer and especially autumn young moles need to make a new territory with their own tunnels. And fourthly, the number of earthworms also influences mole activity. The presence of earthworms is – among other things – influenced by the moisture of the earth, but also by cultivating the land. For example, the fertilizing in spring induces enough vibrations in the earth to cause earthworm migration to shallower ground levels. This migratory behaviour is also known by gulls that search for food on recently cultivated ground. Similar vibrations are also caused by moving animal herds. The latter cause especially could influence the *Salmonella*-prevalence in moles. The active use of meadows could increase mole-activity, which indirectly increases contact at the surface between moles and cattle.

In the end little information about *Salmonella*-prevalence in moles is known. Instead of the moles themselves, it would be interesting to study the containment of molehills bacteriologically. It could be that moles themselves are not infected nor carriers of *Salmonella*. However, it might be present on their fur, or they could dig up some specific pathogens in deeper ground layers and transfer this to the surface. Those molehills could come into direct contact with cattle, or they might end up in the feed during harvesting.

Hares and wild rabbits

Both hares and wild rabbits live on the meadows that feed cattle in Salland. So, direct and indirect contact between those animal groups is possible. In the current study two wild European rabbits (*Oryctolagus cuniculus*) were sampled and declared *Salmonella*-free. The noticeably low number of hares and wild rabbits in the current study is mostly caused by the RHD-virus epidemic during the last two summers, which reduced the population size. Also,

few studies are known to sample hares or wild rabbits for *Salmonella* without being game meat.

Although, the wild rabbits in the study of Vieira-Pinto et al. (2011) are game meat, the samples are faecal. They found a prevalence of 48% (38/80) in Portugal. Türck (2008) mentioned a *Salmonella* prevalence of 12% in the Netherlands and 0-2,55% in Germany in carcasses of hares as game meat.

Evidently, the numbers fluctuate much and being game meat could be the cause. Paulsen et al. (2012) stated that a lot of difference in pathogen prevalence in game meat is caused during the skinning of the carcasses and the way of taking samples. Therefore, based on this information, no conclusion can be made as to whether hares and wild rabbits are a risk for the dairy farm.

Carnivores

Like raptors, the *Salmonella*-prevalence is suspected to be high, because carnivores' position in the food chain. There is little contact between carnivores and Salland's dairy farms' cattle. The current study included 2 samples of red foxes (*Vulpes Vulpes*) and a wild cat (*Felis silvestris*), whose faecal samples were *Salmonella*-free.

In a large study of Nowakiewicz et al. (2016) *Salmonella* prevalence was observed in the three most common carnivores in Poland. This included the red fox with a prevalence of 3,1% (9/286), stone martens (*Martes foina*) with 9,2% (6/65) and raccoons (*Procyon lotor)* with 5,7% (4/70). Nowakiewicz et al. stated that the higher prevalence in stone martens and raccoons is caused by their behaviours. Compared to foxes, stone martens and raccoons live closer to humans, which could cause a higher risk of contracting *Salmonella*. Migration of stone martens to villages in Salland, where they are treated as pests, is becoming more prevalent. On the other hand, Glawischnig, Lazar, Wallner and Kornschober (2017) show a prevalence of 2,1% in foxes in the Tirol-region of Austria. Except for the valleys, this region

has a low human population density, whereas to the south of the Alps – where there is more human interference – two different studies found higher prevalence. In the period of 2002-2010, Botti et al. (2013) cultured 63 *Salmonella*-positive samples out of their 1222 canids samples, and 25 of the 221 mustelids samples. Likewise, Chiari et al. (2014) found a prevalence of 5,7% (29/509) in red foxes in the period of 2009-2010.

The cat that was found in the current study was identified as a wild cat. Normally, cats and dogs are often used as mice and rat catchers on dairy farms in the Netherlands. Earlier in this discussion, it was concluded that mice and rats could maintain the on-farm *Salmonella* infection. Therefore, it seems reasonable to keep cats or dogs. Nevertheless, Skov et al. (2008) found a prevalence 6,4% (3/46) in animal pest catchers on *Salmonella*-positive farms.

To conclude, the prevalence in Dutch foxes is expected to be similar to that found in foxes in northern Italy, namely 5-11%. This expectation is based on the similarity in the human density in both places. Additionally, foxes have been spotted in the outskirts of Dutch cities and villages during the past few years. Nonetheless, the frequency of foxes spotted on farms in Salland is very low, except for in some chicken coops. So, foxes would be considered a low risk factor for introducing *Salmonella* on dairy farms. However, cats and dogs could maintain the on-farm *Salmonella*-infection.

Deer and wild boars

The current study's sample pool is missing samples of deer and wild boars, while they are species recognised in Dutch wildlife. In the Netherlands wild boars only exist in a few nature reserves, while the deer are present in Salland in large numbers.

Deer in Salland are not considered pests, but in some countries in Europe they are (Du Bois, 2013). On agricultural land and orchards, they can cause a lot of damage in times of food shortage and overgrown populations (Lammertsma, Bruinderink and Griffioen, 2012). The relevance of studying deer in relation to dairy farms is supported by the assumption that

diseases spread between ruminants. This might facilitate an easier spread of the bacterium, and thus create a higher risk for a *Salmonella*-infection between wildlife and livestock.

Based on the above, other researchers have observed *Salmonella* prevalence in deer. In Norway, Wahlström et al. (2003) sampled 30 moose (*Alces alces*) and 85 roe -, red – and fallow deer (*Capreolus capreolus*, *Cervus elaphus*, *Dama dama*), none of which tested positive. The same results were seen by Millán et al. (2004) with 19 red - and roe deer samples in Spain. In the neighbouring country, Portugal, Dias et al. (2015) sampled 46 red – and roe deer without a positive *Salmonella*-culture. In the case of Gnat et al. (2015), all cultures were *Salmonella*-negative, taken from 30 red deer from Slovenia, 30 from Hungaria and 60 from Poland. All these results agree with the review of Paulsen et al. (2012), which concludes that *Salmonella* is rarely detected in faeces of wild ruminants.

Unlike in deer, in wild boars (*Sus scrofa*) high prevalence has been found. Millán et al. (2004) found 7,5% (3/40) *Salmonella* prevalence and Wahlström et al. (2003) found 0% (0/31) in their wildlife studies. In studies specific to wild boars the following results can be found: Sannö, Aspan, Hestvik and Jacobson (2014) detected 10% (9/88) prevalence in faecal samples in Sweden; Touloudi et al. (2015) had 4,3% (4/94) serum-samples in Greece; Zottola et al. (2013) had 11% (54/499) in faecal samples and 66,5% (255/383) in serum-samples in Italy; Navarro-Gonzales et al. (2012) had 18% (10/57) in faecal-samples in dairy-free regions of Spain and 36% (57/157) in faecal samples obtained on cattle-grazing areas.

Notably, in comparison to the other animals studied, the wild boar studies used serum-samples. Although a serum-sample can indicate a *Salmonella*-infection, it does not mean the animal excretes *Salmonella*-bacteria into the environment. In cattle, it is known that infected animals test positive to Salmonella-antibodies for at least another 6 months after healing. So,

the expected value of *Salmonella*-excreting animals should be lower than the percentage of sero-positive animals, similar to the study by Zottola et al. (2013).

As a whole, the expected prevalence in the Dutch deer population should be around 0%, based on the aforementioned studies. The risk for dairy farms would be close to none. On the other hand, wild boars would be a real risk for *Salmonella*-introductions. Nevertheless, wild boars will be small risk for dairy farmers in Salland, because of the lack of wild boars in this region.

Conclusion

The biggest risk of *Salmonella*-introduction from wildlife will be presented by carrion birds and gulls. Caused by their non-selective feeding behaviour they have a higher risk to be infected with *Salmonella*. This makes their faecal droppings a high-risk factor to introducing *Salmonella* into dairy farms.

Subsequently, large groups of small garden birds, like sparrows and starlings, are also considered potential *Salmonella*-introducers. The large groups around mating, hatching and migration time pose and exceptionally high risk. However, single individuals and small groups of garden birds are considered to be low-risk.

Attention must be paid to the idea that mice and rats are usually not the *Salmonella*-introducers. Nonetheless, they could present similar issues as on-farm cats and dogs, in that they could maintain the *Salmonella*-infection on the farm as reservoirs. This is an important consideration when making measures to become *Salmonella*-free.

Other species, like raptors, carnivores, deer and wild boars seem to present a low risk for infecting dairy farms in Salland. In the case of moles, hares and wild rabbits, more research should be conducted to gain more insight in the risk those species may present.

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