Salmonella isolates from wild birds and mammals in the Basque Country (Spain)

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Summary
The authors investigated the prevalence of Salmonella spp. in 205 wild birds and mammals belonging to 45 species during the years 2001 and 2002 in the Basque Country (Spain). Salmonella was isolated from 16 (7.8%) animals. The prevalence was 8.5% (7/82) in birds, and 7.2% (9/123) in mammals. Nine serotypes, all of them belonging to the species Salmonella enterica, were identified: two isolates of Typhimurium (from 1/3 griffon vultures [Gyps fulvus], and 1/5 sparrowhawks [Accipiter nisus]); one of 6,14:z4, z23: (subsp. houtenae, 1/1 common kestrel [Falco tinnunculus]); one of Muenchen (1/1 captive Harris’s hawk [Parabuteo unicinctus]); two of Enteritidis (1/5 tawny owls [Strix aluco], and 1/14 foxes [Vulpes vulpes]); one of Give, Newport and Umbilo and one untyped isolate (4/22 badgers [Meles meles]); two of Worthington and one of 38:IV:z35 (subsp. arizonae, 3/40 wild boars [Sus scrofa]); and three other untyped isolates (1/1 northern fulmar [Fulmarus glacialis], 1/11 buzzards [Buteo buteo], 1/4 genets [Genetta genetta]). Salmonella isolation was never associated with macroscopic or microscopic lesions. The results of this study confirm the importance of wildlife as a Salmonella reservoir and as a potential risk for humans and livestock.

Keywords

Introduction
Salmonellosis remains an important problem for humans and animals throughout the world (19). In Spain, bacteria of the genus Salmonella cause 85% of cases of food-borne bacterial enteritis in humans (27) and the number of recorded incidents in animals is increasing each year (37). Many of these isolations also show marked antimicrobial resistances (9).

Salmonella has been isolated from a wide variety of wild animals, as described by Mörner in mammals (21), by Morishita et al. in raptors (20) and by Wilson and MacDonald in wild birds in general (41). It is known to cause death in some wild species, mainly small passerines (5, 18). However, the relevance of wild animals in the epidemiology of salmonellosis is their role as healthy carriers of a broad range of Salmonella serotypes, as observed, for example, by Hudson et al. in the United States of America (10) or Refsum et al. in Norway (30). Furthermore, it has been suggested that in many European countries the serotype Typhimurium has established a reservoir in wild birds (14) and hedgehogs (Erinaceus europaeus) (8, 13). Epidemiological and bacteriological evidence indicates that these animals may transmit the infection to humans (8, 32) or livestock (11).

Very little is known about the role of wildlife as a reservoir of Salmonella in Spain. Some isolates have been found in captive raptors and reptiles (37), but there is only a small
amount of data available regarding free-range animals. For example, in the serum samples of European wild boars (Sus scrofa) Vicente et al. (39) found a low seroprevalence of Salmonella antibodies at meaningful titres. The aim of this study, therefore, was to determine the importance of wildlife as a reservoir of Salmonella in the Basque Country (Northern Spain).

Materials and methods

In 2001 and 2002, 205 wild animals belonging to 45 species were submitted to the laboratory of the authors and examined for Salmonella. These included 82 birds from 31 species, and 123 mammals from 14 species (Table I). The animals had been found dead (mainly road-killed) or shot by hunters (most of the ungulates and some of the red foxes [Vulpes vulpes]).

A complete necropsy of each animal was performed. They were measured and weighed, and macroscopic lesions were recorded. Samples of all organs were fixed in 10% formalin and routinely processed for histopathological studies when the condition of the carcass allowed it. A pooled sample (approximately 1 g) of liver, intestine and spleen was homogenised with 9 ml of sterile phosphate-buffered saline in a Stomacher blender. Two enrichment broths, Selenite-Cystine and Rappaport-Vassiliadis, were inoculated with 1 ml and 10 µl of each broth were inoculated onto two plates of selective media, Salmonella-Shigella agar and brilliant green agar, the plates were then incubated at 37°C for 24 h. Colonies of presumptive Salmonella were confirmed by means of appropriate biochemical and serological tests. A subculture of each strain was sent to the Central Veterinary Laboratory (Algete, Madrid) and/or to the Instituto Carlos III (Madrid) for serotyping.

Results

Salmonella was isolated from 16 out of 205 (7.8%) animals (Table I). The prevalence was 8.5% (7/82) in birds, and 7.2% (9/123) in mammals, affecting six avian and four mammalian species. In birds, the highest prevalence was found in the order Falconiformes (14.3%). Members of the order Carnivora (12.5%) and European wild boar (7.5%) were the only mammals infected. Nine serotypes were identified (Table I). All of them belonged to the species S. enterica. Four isolates were not serotyped. Salmonella isolation was never associated with macroscopic or microscopic lesions. Some degree of seasonal aggregation was observed in the incidence of the isolates, which were mainly found in cold months. In raptors, five out of the six cases were observed between December and April. In the case of the carnivore mammals, four out of the six cases were found between November and March.

Discussion

In contrast to Mörner (21), who stated that salmonellosis in mammals is not as frequent as in birds, the results of the present study showed a similar prevalence for both groups. On the other hand, no signs of disease, either macroscopic or histopathologic lesions, were found in any positive animal. This agrees with previous reports (20), and the authors believe that this may be due to the fact that these animals are only asymptomatic Salmonella carriers.

Birds

With the exception of the northern fulmar (Fulmarus glacialis), all isolations were obtained from raptors, most of which belonged to the order Falconiformes. One of them was a captive Harris's hawk (Parabuteo unicinctus). Salmonella has been isolated occasionally in raptor breeding facilities (1) and such cases may be the result of feeding the birds with quails or pigeons (40).

In free-living raptors, Salmonella has been isolated sporadically in many European raptorial species (15, 30, 41). However, Salmonella epizootics occur much less frequently among raptors than among other avian species (15, 41). Euden (7) reported a total lack of isolations from 78 raptors; Keymer (15) described a prevalence of 1.9% in a sample of 51; and Kirkpatrick (16) also only found 1.9% positive cases among 105 raptors. Therefore, the results of the present study show a high prevalence when compared with previous reports.

Four serotypes were found in raptors in this study. Salmonella Typhimurium was found in a griffon vulture (Gyps fulvus) and a sparrowhawk (Accipiter nisus). This serotype has been previously described in barn owls (Tyto alba) nesting near farms and houses (17), and is very frequently found in Spanish livestock (4). This serotype was almost the only one found among 470 isolations from wild birds in Norway (30). It has also been reported in captive raptors (40). Moreover, it is frequently isolated from human samples in Spain (3).

Salmonella Enteritidis, found in a tawny owl (Strix aluco), was the serotype most frequently isolated from human, food and environmental sources in Spain in 2000, and it was also frequent in birds (36, 37). Salmonella Muenchen, isolated from the captive Harris's hawk, was also reported from human and animal sources in Spain in 2000 (36, 37).
Table I
Salmonella isolations in wild birds and mammals in the Basque Country (Spain) in 2001-2002

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of animals examined</th>
<th>Isolates</th>
<th>Prevalence (%)</th>
<th>Serotypes</th>
<th>Free of Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procellariiformes</td>
<td>82</td>
<td>7</td>
<td>8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern fulmar</td>
<td>1</td>
<td>1</td>
<td>100.0</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Ciconiformes</td>
<td>9</td>
<td></td>
<td></td>
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<tr>
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<td>1</td>
<td>1</td>
<td>100.0</td>
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<td>yes</td>
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<tr>
<td>Grey heron</td>
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<td>1</td>
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</tr>
<tr>
<td>White stork</td>
<td>3</td>
<td>1</td>
<td>33.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gruidiformes</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common moorhen</td>
<td>1</td>
<td>1</td>
<td>100.0</td>
<td></td>
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<tr>
<td>Caprimulgiformes</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Nightjar Caprimulgus europaeus</td>
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<td>Falconiformes</td>
<td>35</td>
<td>5</td>
<td>14.3</td>
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<tr>
<td>Griffon vulture Gyps fulvus</td>
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<td>1</td>
<td>33.3</td>
<td>Typhimurium</td>
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<tr>
<td>Sparrowhawk Accipiter nisus</td>
<td>5</td>
<td>1</td>
<td>20.0</td>
<td>Typhimurium</td>
<td></td>
</tr>
<tr>
<td>Buzard Buteo buteo</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
<td>n.s.</td>
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<tr>
<td>Common kestrel Falco tinnunculus</td>
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<td>1</td>
<td>100.0</td>
<td>6,14:z4,z23:</td>
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<tr>
<td>Harris's hawk Parasuteo unicinctus</td>
<td>1</td>
<td>1</td>
<td>100.0</td>
<td>Muenchen</td>
<td></td>
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<tr>
<td>Red kite Milvus milvus</td>
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<td>1</td>
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<tr>
<td>Black kite Milvus migrans</td>
<td>6</td>
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<tr>
<td>Goshawk Accipiter gentilis</td>
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<td>Honey buzzard Pennis apivorus</td>
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<tr>
<td>Hobby Falco subbuteo</td>
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<tr>
<td>Peregrine falcon Falco peregrinus</td>
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<td>2</td>
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<tr>
<td>Strigiformes</td>
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<td>1</td>
<td>5.3</td>
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<tr>
<td>Tawny owls Strix aluco</td>
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<td>1</td>
<td>20.0</td>
<td>Enteritidis</td>
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<td>Barn owl Tyto alba</td>
<td>8</td>
<td>1</td>
<td>yes</td>
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<tr>
<td>Long-eared owl Asio otus</td>
<td>4</td>
<td>2</td>
<td>yes</td>
<td></td>
<td></td>
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<tr>
<td>Eagle owl Bubo bubo</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td></td>
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<tr>
<td>Little owl Athene noctua</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td></td>
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<tr>
<td>Galliformes</td>
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<tr>
<td>Red-legged partridge Alectoris rufa</td>
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<td></td>
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<tr>
<td>Charadriformes</td>
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<tr>
<td>Lapwing Vanellus vanellus</td>
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<tr>
<td>Common murre Unia aalge</td>
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<tr>
<td>Stone curlew Burhinus oedicnemus</td>
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<td>1</td>
<td>yes</td>
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<tr>
<td>Passeriformes</td>
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<tr>
<td>Magpie Pica pica</td>
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<tr>
<td>Carrion crow Corvus corone</td>
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<td>5</td>
<td>yes</td>
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<tr>
<td>Song thrush Turdus philomelos</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td></td>
<td></td>
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<tr>
<td>Blackcap Sylvia atricamilla</td>
<td>1</td>
<td>1</td>
<td>yes</td>
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<tr>
<td>Spotless starling Stundus unicolor</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td></td>
<td></td>
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</tbody>
</table>

Serotype 6,14:z4,z23: (which belongs to subsp. IV or houtenae), which was isolated from a common kestrel (Falco tinnunculus) (Table I), had been reported in wild opossums (Didelphis virginiana) (31) and in cockateels (28) outside Spain, but inside the country it had only previously been isolated from environmental waters (37).

Mammals

As observed in raptors, the prevalence of Salmonella infections found in mammals in the present study is higher than those previously reported (7, 12, 42). Isolations were obtained exclusively from carnivores and wild boars. With the exception of the genet (Genetta genetta), all the species had been previously reported as carrying Salmonella (7, 13, 39).

Five serotypes were found in mammals. A fox carried 5. Enteritidis, which, as stated above, is frequently isolated in Spain. Three different serotypes (Newport, Give and Umbilo) were found in the three positive badgers. This agrees with previous reports (42). These authors stated that the variety of the serotypes is probably a reflection of the badgers very varied diet. These three serotypes were not frequently isolated from animal or human sources in Spain in 2000, although, in the case of swine, S. Give was relatively frequent (36, 37). Serotype Umbilo has also been found in human and swine sources in Spain in previous years (34, 35). However, isolations in Spain are principally connected with environmental waters which could have been contaminated by human sewage (29); water plays an important role in the spread of the organisms to man and animals (22). Neither Wray et al. (42) nor Euden (7) reported any of these serotypes in British badgers, Agama being the most frequently isolated in both studies.

Two serotypes were isolated from wild boars, two serotypes were isolated: Worthington and 38:IV:z35. The first was isolated from humans and foodstuffs such as chicken carcases in Spain in 2000 (2, 38), but not from other animal sources. This serotype has also been found in the faeces of pigs (6), and from litter and feeder contents from a turkey (Meleagris gallopavo) farm (23). Serotype 38:IV:z35 belongs to subsp. IIIb or arizonae, which has been previously reported in poultry and sheep (24). Usera et al. (37) stated that outbreaks due to Salmonella subspecies other than enterica may be related to exotic reptiles being used as pets. For example, Oros et al. (25) reported an infection due to this subspecies in a captive Honduran milk snake (Lampropeltis hondurensis) in Spain. Salmonella recorded by Vicente et al. (39) in wild boars in central and southern Spain belonged to different serogroups (B and C) from those found in this study.
Seasonality

The authors found that, both in raptors and carnivore mammals, the greatest number of isolations was obtained from animals that were submitted to the laboratory between November and April. Some of the analysed birds are migratory, and this could bias the results. However, the results of the present study agree with the general belief about Salmonella outbreaks occurring during winter months (10, 18). For example, Pennycott et al. (26) reported that 95% of deaths from salmonellosis occurred between January and April, although positive faecal samples were also found throughout the year. Therefore, the animals found infected in this study could have acquired the infection when preying upon small passerines.

Only the isolations from the griffon vulture, the fox and one of the badgers were obtained from animals submitted to the laboratory outside these months, suggesting that these animals could have acquired the bacteria from other sources such as, for example, livestock carcasses or human residues.

Conclusions

All isolates found in the present study were obtained from carnivore, piscivore or scavenger animals. The behaviour and feeding habits of animals certainly influences the likelihood of their being infected with Salmonella (7). This may explain the absence of isolations from, for example, cervids or lagomorphs.

As stated above, the griffon vulture and the wild boars, and probably the fox and some badgers, could have acquired Salmonella by scavenging on contaminated carcasses or different sources of human foodstuff residues. In fact, Euden (7) thought that the origin of some of the Salmonella isolates from British badgers could have been livestock farms.

The raptors and some of the carnivores may have acquired the bacteria by ingesting small passerines or mice. As observed by Kirkpatrick and Trexler-Myren (16), finding Salmonella in raptors may indicate infection in other animal populations (e.g. small mammals and passerine birds). For example, Keymer (15) described a Salmonella isolate from a tawny owl and from the viscera of two mice found in its gizzard. Small passerines, mainly those feeding on garden feeders, are particularly prone to the infection (26). Surveys conducted in Norway have shown that Salmonella is endemically present in Norwegian avifauna, and that small passerines act as healthy carriers of this bacterium (30). Pigeons (Columba livia), frequently preyed upon by raptors, could also act as a reservoir of this bacteria, particularly of serotype Typhimurium (33). Unfortunately, few of these animals were analysed in the present work.

It can be concluded that wildlife is an important Salmonella reservoir and may be a risk for human and livestock. However, the absence of phage typing or the use of molecular genetic techniques make it difficult to draw further conclusions about the role of these isolates in the epidemiology of salmonellosis in humans. More research is needed in order to obtain more detailed information on the epidemiology of Salmonella in the Basque Country.

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Isolats de *Salmonella* chez des oiseaux et des mammifères sauvages du Pays basque espagnol

J. Millán, G. Aduriz, B. Moreno, R.A. Juste & M. Barral

**Résumé**

En 2001-2002, les auteurs ont étudié la prévalence de *Salmonella* spp. chez 205 oiseaux et mammifères sauvages de 45 espèces dans le Pays basque espagnol. Des salmonelles ont été isolées chez 16 animaux (7,8 %). La prévalence était de 8,5 % chez les oiseaux (7/82) et de 7,2 % chez les mammifères (9/123). Un total de neuf sérotypes appartenant à l’espèce *Salmonella enterica* ont été identifiés : deux isolats du sérotype Typhimurium chez un vautour fauve *Gyps fulvus* (1/3) et chez un épervier d’Europe *Accipiter nisus* (1/5) ; un isolat du sérotype 6,14:z4, z23: (subsp. houtenae) chez un faucon crécerelle *Falco tinnunculus* (1/1) ; un isolat du sérotype Muenchen chez une buse de Harris *Parabuteo unicinctus* (1/1) ; deux isolats du sérotype Enteritidis chez une chouette hulotte *Strix aluco* (1/5) et un renard *Vulpes vulpes* (1/14) ; un isolat des sérotypes Give, Newport et Umbilo ainsi qu’un isolat non typé chez des blaireaux *Meles meles* (4/22) ; deux isolats du sérotype Worthington et un isolat du sérotype 38:IV:z35 (subsp. arizonae) chez des sangliers *Sus scrofa* (3/40) ; trois autres isolats non typés chez un fulmar boréal *Fulmarus glacialis* (1/1), une buse variable *Buteo buteo* (1/11) et une genette *Genetta genetta* (1/4). L’isolement de *Salmonella* n’était jamais associé à la présence de lésions macroscopiques ou microscopiques. Les résultats de cette étude confirment le rôle des animaux sauvages en tant que réservoir important de *Salmonella* et le risque potentiel qu’ils constituent pour l’homme et le bétail.

**Mots-clés**


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Cepas de *Salmonella* aisladas en aves y mamíferos salvajes del País Vasco (España)

J. Millán, G. Aduriz, B. Moreno, R.A. Juste y M. Barral

**Resumen**

Durante los años 2001 y 2002, los autores investigaron la prevalencia de *Salmonella* spp. en 205 aves y mamíferos salvajes pertenecientes a 45 especies del País Vasco (España). Se aislaron cepas de *Salmonella* en 16 animales (7,8%). La prevalencia era del 8,5% (7/82) en las aves y del 7,2% (9/123) en los mamíferos. Se identificaron nueve serotipos de la especie *Salmonella enterica*: dos cepas de Typhimurium (en 1/3 de buitres comunes *Gyps fulvus* y 1/5 de gavilanes *Accipiter nisus*); una de 6,14:z4, z23: (subespecie houtenae, en 1/1 cernicalo vulgar *Falco tinnunculus*); una de Muenchen (en 1/1 gavilán mixto *Parabuteo unicinctus* en cautiverio); dos de Enteritidis (en 1/5 de cárabos comunes *Strix aluco* y en 1/14 de zorros *Vulpes vulpes*); una de Give; Newport y Umbilo y una cepa atípica (en 4/22 de tejones *Meles meles*); dos de Worthington
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