Network for wildlife health surveillance in Europe

Diagnosis Card

Salmonellosis

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Aetiology

Salmonella spp. are facultative anaerobic, rod-shaped, Gram-negative bacteria. *Salmonella* is a virtual universal and robust pathogen that has demonstrated remarkable adaptability to survive in varying environmental conditions. *Salmonella* exhibits notable resistance to changes in temperature and pH, with some strains capable of enduring extremes. *Salmonella* generally grows in the temperature range of 10-47 °C and at pH values between 4 and 9. *Salmonella* has shown the capacity to persist for extended periods in dry environments, such as desiccation.
The genus *Salmonella* comprises two species, *Salmonella* (*S.) enterica* and *Salmonella bongori. Salmonella enterica* is further subdivided into six sub-species such as *S. enterica subspecies I (enterica), II (salamae), IIIa (arizonae), IIIb (diarizonae), IV (houtenae)* and *VI (indica)*. Recent genome-based studies even propose the existence of eleven sub-species. Both species encompass approximately 2,700 serovars. *S. bongori* and the subspecies II, IIIa, IIIb, IV and VI are primarily associated with cold-blooded animals and are considered to have low clinical relevance. *S. enterica* subsp. *enterica* (I.) is, however, responsible for approximately 99% of human cases of Salmonellosis. Serovars of subspecies *enterica* exhibit significant variation in their host spectrum and vary in their clinical manifestations. Serovars can be host-restricted (e.g. *S.* Typhi in humans, *S*. Gallinarum in poultry), host-adapted (e.g. *S.* Choleraesuis in swine, *S*. Dublin in cattle) or have a broad host range (e.g. *S*. Enteritidis, *S*. Typhimurium).

Affected species (wildlife, domestic animals, humans)

Salmonella is a zoonotic pathogen mainly transmitted to humans *via* contaminated food. *Salmonella* is the second most frequent cause of human gastrointestinal infections in the EU and the main reason for foodborne outbreaks, with *S*. Enteritidis and *S*. Typhimurium playing the most important role in human infections. The main reservoirs for *Salmonella* spp. are wild and domestic animals, such as livestock and pets. Livestock animals, especially poultry and swine, are common carriers of *Salmonella* which colonize their gut. Animals can transmit the bacterium to humans by direct contact, but also *via* entry to the food chain at various food processing points. *Salmonella* is widespread in the environment and is also known to colonise and internalize plants.
Infections caused by close or direct contact with wildlife, or their natural habitats are seldomly described. The reported cases affect mainly children, due to their contact with petting zoos and reptiles, such as turtles or lizards, which can be potential sources of *Salmonella* infections. Children, especially toddlers, are at a higher risk because they commonly neglect any hygiene practices and often put their hands in their mouths after having had contact with animals. However, in addition to infections linked to consumption of wildlife-derived food products, such as fresh sausages, salami and undercooked liver, wildlife can also contaminate crop plants and domestic animal housings. In-depth genetic comparisons revealed that wildlife species could carry highly similar isolates also found in infected humans, foodstuff, and livestock animals, thus highlighting a likely circulation of *Salmonella* clones between the different compartments.
Salmonella spp. have been isolated from birds, rodents, foxes, deer, hedgehogs, wild boars, reptiles, seals, elephants, and other wild species. In a few species, an association with specific *Salmonella* genetic lineages belonging to a certain sequence type has been demonstrated, such as *S*. Typhimurium ST128 in pigeons, *S*. Enteritidis ST183 in hedgehogs, or *S*. Choleraesuis ST145 in wild boars.

Epidemiological characteristics and disease course

The prevalence of *Salmonella* in wildlife can vary seasonally and by geographic location and is

influenced by factors such as climate and the dynamics of the wildlife population. Wild animals can be asymptomatic carriers of *Salmonella* without showing clinical symptoms and even without shedding the bacteria. However, stress factors such as changes in habitat, food availability or human disturbance can trigger the pathogen shedding. Host-adapted serovars are more likely to occur in certain wildlife species, such as *S*. Choleraesuis in wild boars.
A *Salmonella* infection usually begins with colonisation of the pathogen in the intestine followed by adhesion and invasion of the enterocytes, resulting in inflammatory reaction and diarrhoea. *Salmonella* can also spread to the lymph nodes and organs. Fatal septicaemic salmonellosis has also been reported in humans and in animals.
Clinical signs usually last for 2-7 days but death can occur within 24-48 hours in some species.

Clinical signs

Acute *Salmonella* infections can result in clinical symptoms such as fever, diarrhoea, lack of appetite, vomitus, apathy and even death. Furthermore, abscesses, organ inflammations, tendinitis and abortion may occur. Stress factors can exacerbate the course of salmonellosis. Chronic salmonellosis is mainly symptomless, whereas animals became long-time carriers/reservoirs of *Salmonella*. In carrier animals, the bacteria often colonise the intestine and are temporarily excreted in the faeces.
Most of the infected animals do not show clinical symptoms. Therefore, clinical salmonellosis is only rarely reported in wild animals. One exception is however the salmonellosis of wild songbirds. Infection occurs during the winter months, when bacteria are spread through infected animals, especially while sharing bird feeders. Typical signs are weakness and ruffled feathers; mortality is high, sometimes even without clinical signs.
Salmonellosis of wild boars related to *S.* Choleraesuis has also been reported; however, the diagnosis was made post-mortem in absence of clinical signs.
In farmed wild boars, progressive weight loss, anorexia, weakness, difficulties in walking, lethargy, and death within three to five days after the onset of symptoms are described. Also, death without clinical signs was observed.

Gross lesions

The enteric form of salmonellosis often has no mucosal lesions of the intestine, only a thin liquid content, oedematous swelling of the lymph nodes and sometimes signs of endotoxin shock. In the septicaemic form of salmonellosis, hyperaemic splenic swelling, congestive hyperaemia, catarrhal to ulcerative enteritis, lymphadenitis of the intestinal lymph nodes, myocarditis, nephritis, and hepatitis can be observed. The course of septicaemic disease is often accompanied by joint swelling, but meningitis has also been diagnosed.

Histological lesions

Histological lesions of salmonellosis in asymptomatic carriers can be absent. The reported histopathological lesions in wild animals include haemorrhages, microthrombi and necrotic foci in internal organs and lymph nodes, hyperplasia of lymphoreticular tissue, interstitial pneumonia, oedema, ulcerative typhlocolitis and ileitis with typical button ulcers.

Differential diagnosis

- Enteric infections or intoxications of different aetiology (e.g., *Clostridium perfringens*, *Escherichia coli*, Endoparasites)
- Septicaemia of different aetiology (e.g., *Escherichia coli, Pseudomonas* spp.).
- Abortion of different aetiology

Criteria for diagnosis

Both medical history and clinical signs are indicative only of a diagnostic suspicion. The final diagnosis must be confirmed by direct detection of the pathogen (bacterial culture from faeces, organs, or blood).

Recommended diagnostic method(s) and preferred samples

The detection of *Salmonella* spp. should be performed according to the currently valid version of ISO 6579-1: Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.
The preferred samples are faecal samples, either individual or pooled. In case of dead and hunted animals, or carcasses with signs of septicaemic disease, organ samples such as mesenterial lymph nodes, tonsils, spleen, liver, lung, intestine, and blood can be analysed. In case of abortion, aborted material and foetus can be analysed. For epidemiological purposes, feed and environmental samples should also be tested. Preferably freshly collected material should be used for testing.
The detection procedure according to DIN EN ISO 6579-1 in the currently valid version includes the following steps:

- Pre-enrichment in Buffered Peptone Water (18 h ± 2 h at 34°C to 38°C)
- Selective enrichment in Modified Semi-solid Rappaport Vassiliadis (MSRV) medium

The semi-solid medium added with inhibitory compounds suppresses the growth of the background flora and enables the multiplication and swarming of *Salmonella* on the surface of the plates. The MSRV medium is inoculated with 0,1 ml of the pre-enriched culture in three equally spaced spots on the surface of the plate. MSRV medium is incubated two times 24 h ± 3 h at 41,5 °C ± 1 °C.

- Inoculation of two selective isolation agar media

A loop of material from the outer edge of the swarming zones on MSRV is streaked onto two solid selective media, to obtain well isolated single colonies. The first isolation medium is XLD and the second is chosen by the laboratory. The choice of the medium depends largely on the material to be examined and therefore on the expected background flora. Selective media are incubated at 37 °C ± 1 °C for 24 hours.

- Confirmation by biochemical and serological examination

Typical or suspect colonies are subcultured on a not selective agar media (e.g. Tryptone Soya Agar) for confirmation. The combination of biochemical and serological (serotyping) test results indicates whether an isolate belongs to the genus *Salmonella*. Alternative methods for confirmation, e.g. real-time PCR, are widely used.

The isolates that are confirmed as *Salmonella* spp. can be further typed to serovar level according to the White-Kauffmann-Le Minor scheme. Guidance for serotyping is described in ISO/TR 6579-3: Horizontal method for the detection, enumeration and serotyping of *Salmonella* –Part 3: Guidelines for serotyping of *Salmonella* spp.

Alternative methods for detection of the pathogen (e.g. enzyme-linked immunosorbent assay (ELISA), PCR and real-time PCR assays, impedance procedure, next generation sequencing metagenomic) are also available. However, the criteria for diagnosis should be direct isolation of the bacteria.

Laboratories that can be contacted for diagnostic support

- Reference Laboratories for Salmonellosis of the WOAH https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/
- Institutes for Veterinary Microbiology
- National *Salmonella* Reference Laboratories

Recommended literature

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