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Review

The genus *Salmonella*, isolation and occurrence in wildlife

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The genus *Salmonella* belong to the family *Enterobacteriaceae* and tribe *Salmonelleae*. It has only two species, *Salmonella enterica* and *Salmonella bongori*. The later is further subdivided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. The number of serotypes in each species and subspecies of *Salmonella* is reported to be around 2522. Different transport/pre-enrichment media, enrichment media, differential and selective solid media, biochemical tests, serological tests and biotechnological procedures are used in the isolation of *Salmonella*. Although published reports on the isolation of *Salmonella* in wildlife are few, the organism had been isolated from some wild animals which include elephants, giraffes, rhinoceros, chimpanzee, etc. However, published works were used mainly as the data base of the study.

Key words: *Salmonella enterica, Salmonella bongori*, wildlife, salmonellosis.

THE GENUS *SALMONELLA*

Edwards and Ewing initiated the work to define and identify strains of the genus *Salmonella* at the Centre for Disease Control (CDC) in the late 1940s, although over the years, taxonomy and nomenclature have changed and are still evolving, but many of the methods they developed and described are still in use (Ewing, 1986).

The genus *Salmonella* is composed of motile bacteria which conform to the definitions of the family *Enterobacteriaceae* and tribe *Salmonelleae*. Hydrogen sulphide is produced, methyl red reaction is positive, lysine and ornithine are decarboxylated, arginine is dehydrolysed, indole is not formed, urea is not hydrolysed, Voges – Proskauer test is negative and neither phenylalanine nor tryptophan is deaminated. Acid is not produced from sucrose, adonitol, raffinose, or alpha – methylglucoside. Lactose is fermented by most strains belonging to subspecies IIIa and IIIb but not by those of subspecies I, II, IV or V. Dulcitol is fermented by members of subspecies I, II and V but not by those of IIIa, IIIb or IV. Inositol is not fermented by strains of subspecies IIIa, IIIb, IV or V (Ewing, 1986).

Subspecies VI was later described by Le Minor et al. (1986) consisting of strains that are inositol and sorbitol negative, with 22% fermenting lactose and 67% fermenting dulcitol.

Subspecies V was reported to be distinct from the other species of *Salmonella* and in 1989 it was proposed that subspecies V should be placed in a second species which is designated as *S. bongori* (Reeves et al., 1989). Thus, the genus *Salmonella* currently has two species: *S. enterica* and *S. bongori* (Table 1).

S. enterica is further divided into six subspecies: *S. enterica* subspecies *enterica* (designated I or 1), *S. enterica* subspecies *salamae* (II or 2), *S. enterica* subspecies *arizonae* (IIIa or 3a), *S. enterica* subspecies *diarizonae* (IIIb or 3b), *S. enterica* subspecies *houtenae* (IV or 4), *S. enterica* subspecies *indica* (VI or 6).

The other species, *S. bongori*, was formerly subspecies V or 5 (McWhorter - Murlin and Hickman - Brenner, 1986).

The legitimate species name for strains of subspecies I - IV and VI is *S. choleraesuis* and not *S. enterica*, but

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Table 1. The current number of *Salmonella* serotypes in each species and subspecies.

Serotype name	Number
S. enterica	**2,501**
Subsp. *enterica* (I)	1478
Subsp. *salamae* (II)	498
Subsp. *arizonae* (IIIa)	94
Subsp. *diarizonae* (IIIb)	327
Subsp. *houtenae* (IV)	71
Subsp. *indica* (VI)	12
***S. bongori* (V)**	**21**
Total	2,522

Source: Popoff (2001).

because it can be confused with the common serotype that is also designated 'choleraesuis', the international subcommittee for Enterobacteriaeae unanimously agreed to adopt the species name *S. enterica* (Penner, 1988).

Majority of the *Salmonella* serotypes belong to subspecies I or the *enterica* subspecies.

For example, most *Salmonella* in the first nine O groups that is A, B, C1, C2, D, E1, E2, E3 and E4 and most *Salmonella* strains isolated in clinical laboratories which include *S. enterica* subspecies *enterica* serotype Typhimurium or *Salmonella* serotype (or ser.) Typhimurium, or *S.* Typhimurium, *S*. Choleraesuis, *S*. Enteritidis. Subspecies I strains are usually isolated from humans and warm blooded animals (McWhorter and Hickman, 1986).

Antigenic formulas of *Salmonella* serotypes are listed in a document called the Kauffmann – White scheme which is updated by the WHO Collaborating Centre for Reference and Research on *Salmonella* located at the Pasteur Institute, Paris, France (Popoff and Le Minor, 1992; Popoff et al., 1993).

Palmgren et al. (2006) also reported that there are more than 2,500 serovars or serotypes of this bacterium known.

Serotypes belonging to *Salmonella* subspecies I are usually designated by a name related to the geographic location where the serotype was first isolated with the serotype name written in Roman letters, not italicized and the first letter is a capital letter. For example, *Salmonella* serotype (abbreviated ser.) Typhimurium (McWhorter and Hickman, 1986), *Salmonella* ser. Zaria (Kwaga et al., 1985).

Serotypes could also be designated by their antigenic formulas for example *Salmonella enterica* subsp. *diarizonae* serotype 60:k:z or abbreviated *S*. serotype 60:k:z and *Salmonella enterica* subsp. *enterica* serotype 17:k:e,n,z15 which is the antigenic formula for *Salmonella* Zaria (Kwaga et al.,1985b; McWhorter and Hickman, 1986).

ISOLATION OF *SALMONELLA*

Salmonella transport/pre - enrichment media

These are used to support the life of the bacteria especially if the samples will have to be transported over a distance and for a period of time. Abundant growth with uniform turbidity is achieved. These include:

1. Peptone water;
2. Tryptone soya broth;
3. Nutrient broth (Cruickshank et al., 1975);
4. Rappaport-Vassiliadis (RV) (Waltman, 2000).

Salmonella enrichment media

These are liquid media used to assist in the isolation of *Salmonella* from faeces, sewage and other materials with mixed bacteria flora. They aid the growth of *Salmonella* while limiting that of *E. coli* and other organisms before plating on solid media. These include:

1. Tetrathionate broth, with or without brilliant green which increases the selectivity of *Salmonella* but is rather too inhibitory for *S.* Typhi.
2. Selenite - F broth is the most used enrichment medium for *Samonella* isolation. Hurley and Ayres (1953) compared the use of six enrichment media and recommended the use of selenite F broth among others.
3. Strontium chloride broth was found by Iveson and Mackay-Scollay (1969) to be superior to selenite - F broth especially for the isolation of *S.* Typhi.
4. Selenite – M.
5. Ruys' medium.
6. Rappaport – Vassiliadis.
7. ¼ Ringer's solution (Hurley and Ayres, 1953).

Salmonella differential and selective solid media

These are valuable for the isolation of *Salmonella* from faeces and other materials that may be contaminated with many bacteria of other kinds. Aerobic and facultative anaerobic bacteria grow on simple laboratory media at a temperature range of 15 - 41°C, optimally at 37°C (Stokes and Bayne, 1958). The different culture media used for the isolation of *Salmonella* include the following:

(i) MacConkey's bile salt lactose agar medium in which *Salmonella* colonies are pale yellow or nearly colourless after 18-24 h at 37°C. The colonies are 1-3 mm in diameter and easily distinguished from the pink - red colonies of the lactose fermenting coliform bacilli which grow well also on this unselective, differential medium.
(ii) The addition of 0.004% brilliant green to brilliant green MacConkey agar medium is inhibitory to *Escherichia coli* and other enterobacteria likely to outnumber *Salmonella* in faeces and makes the medium selective for *Salmonella*

except for *S.* Typhimurium.
(iii) On Leifson's Deoxycholate - Citrate Agar (DCA), *Salmonella* colonies are pale to nearly colourless, smooth, shiny, translucent with or without black centers and are slightly smaller in size compared to those on MacConkey agar. They are easily distinguished from the opaque pink colonies of lactose fermenting coliform bacillus which are largely inhibited by the medium.
(iv) Wilson and Blair's brilliant green Bismuth Sulphite Agar (BSA) is particularly viable for the isolation of *S.* Typhimurium. *Salmonella* colonies appear closely packed about 1 mm in diameter appearing greenish or pale brown with or without black centers after 24 - 48 h. This medium is highly selective for *Salmonella* (Cruickshank et al., 1975).
(v) *Salmonella* colonies appear colourless with or without black centers on Salmonella - Shigella agar (SSA).
(vi) They appear blue green with or without back centers on Hektoen agar; black or red or pink with or without black centers on Xylose Lysine Tergitol 4 (XLT4). They appear red with or without black centers on Xylose Lysine Deoxycholate (XLD) agar and yellow on Gassner agar (Waltman, 2000).

CONFIRMATORY TESTS

Biochemical reactions

Carbohydrates are fermented by *Salmonella* with the production of acids and/or gas. *S.* Typhimurium, *S.* Gallinarum and *S.* Newport for example form only acid.

Typically, *Salmonella* ferment glucose, mannitol, arabinose, maltose, dulcitol and sorbitol while lactose, sucrose, salicin and adonitol are not fermented. ONPG test is negative (Cruickshank et al., 1975).

Pure single/discrete colonies suspected to be *Salmonella* are picked from the agar plates for fermentation tests. Usually triple sugar iron (TSI) agar containing glucose, lactose, sucrose, ferrous sulphate and phenol red indicator is used. When any of the three sugars are fermented, the colourless medium turns yellow but if it is only glucose that is fermented, red (alkaline) colouration is observed on the slants especially under aerobic conditions and because of protein breakdown. Underneath the tube, in an anaerobic condition, the medium remains yellow (acid). H_2S production is indicated by the blackening of the medium (Jones et al., 2000).

Colonies which produce characteristic *Salmonella* results in TSI are inoculated into urea agar. Urease is not produced by *Salmonella*, that is, *Salmonella* is urease negative. *Salmonella* decarboxylates amino acid lysine, ornithine and arginine but not glutamic acid.

In other biochemical tests, indole is not produced, methyl red is positive, Voges - Proskauer is negative, H_2S may or may not be produced in ferrous chloride gelatin medium (Cruickshank et al., 1975).

SEROLOGICAL TEST

Presumptive *Salmonella* colonies on the solid agar that are TSI positive and urea negative are further tested serologically with polyvalent antiserum for *Salmonella*. One of the most commonly used is the polyvalent antiserum containing agglutinins for the 'O' antigen of serological groups A - E. About 95% of *Salmonella* serotypes from man and lower animals belong to these few serogroups. This is done by suspending a loop full of the growth from TSI or from XLD agar in one or two drops of normal saline and mixing with one drop of the polyvalent antiserum on a glass slide after which the slide is tilted back and forth several times. Positive tests are indicated by rapid, complete agglutination of the bacterial cell (Ewing, 1982; Yan et al., 2003). Other antisera that could be used are:

i) Group specific antisera.
ii) Poly H *Salmonella* antisera.

Salmonella Microbact GNB 12E testing

Microbact GNB 12E is a computer aided identification system with readings taken based on colour change due to substrate utilization, pH change and comparison with the provided standard colours. The colour changes are scored and the scores entered into the software provided for percentage identification of the organism (Mugg and Hill, 1981).

Mugg and Hill (1981) compared the Microbact 12E and 24E systems and the API - 20E system for the identification of Enterobacteriaceae and reported that the Microbact system is accurate, convenient and easy to use as compared to the conventional biochemical tests.

Molecular diagnosis (Polymerase chain reaction, PCR) for *Salmonella*

The gene which codes for virulence in *Salmonella*, *inv* A gene is what is targeted in this procedure. Primers produced to target this region are used which, through complimentary base pairing, anneal to the *inv* A target sequence. With the presence of *Taq* polymerase, the DNA strands are copied and amplified (Van der Zee and Huis, 2000).

After DNA extraction, *inv* A forward and reverse primers, *Taq* polymerase, dNTPs, $Mgcl_2$ and water are mixed and put in a thermal cycler for the PCR to take place. The three steps of denaturization, annealing and elongation are usually repeated in about 40 cycles, analysis of the amplified DNA is then done with agarose gel electrophoresis (Al-Gallas et al., 2002).

OCCURRENCE OF *SALMONELLA* IN WILDLIFE

Salmonella are found in many species of animals

including birds, reptiles, man and aquatic animals where they often cause diseases, acute and chronic diarrhea and deaths (Mcgavin et al., 2001). Despite this, reported cases of *Salmonella* infection in wild animals outside and within Africa are few; and the documented works are not recent (Gitter and Brand, 1969; Falade and Durojaiye, 1976; Okoh and Onazi, 1980).

Salmonella species have assumed increased significance due to their ubiquitous distribution, the growing number of serotypes, wide host range (including wildlife), complex pathogenesis, and complicated epizootiology involving humans, domesticated and wild animals and the environment (Morse and Duncan, 1974; D'Auoust, 1989; Moustafa, 1989).

The bacteria also inhabit the intestinal tract of vertebrate and invertebrate animals worldwide and various carrier states have been recognized. The carrier state is the major source of infection for animals and humans. Excretion of the organism results in the contamination of water, food and the environment with wildlife animals playing important roles (Wray and Sojka, 1977; Turnbull, 1979).

Salmonella have been isolated from a number of wild animals (Palmgren et al., 2006). In a survey of wild animals including deers, hares, moose, wild boars, Canada geese and gulls in Sweden in 1999, only gulls were positive for *Salmonella* (Anon, 2000).

Further studies focusing on wild birds as carriers of *Salmonella* have shown that wild birds which live close to humans and feed on human garbage or sewage are more likely to contract *Salmonella*. Wild birds spreading enteropathogenic bacteria to food and shelters of domestic animals, or food processing plants like dairies are suggested as a risk factor for human and domestic animal salmonellosis (Koplan, 1978; Reilly, 1981).

Previous studies have pointed to gulls, especially the black headed gulls (*Larus ridibundus*) as the most important wild bird *Salmonella* reservoir in Europe and *S.* Typhimurium as the most common serotype found in wild birds (Hatch, 1996; Palmgren et al., 1997; Hernandez et al., 2003). *Salmonella* infection in the black-headed gulls was found to be of short duration and expressed predominantly as carriage without disease manifestation (Palmgren et al., 2006).

In a research work carried out by Jones and Twigg in Great Britain, a total of 1,269 wild animals which included 364 house mice (*Mus musculus*), 1 brown rat (*Rattus norvegicus*), 114 wood mice (*Apodemus syvaticus*), 110 bank voles (*Clethrionomys glareolus*), 25 short tailed voles (*Microtus agrestic*), 26 grey squirrels (*Sciurus spp*), 404 brown hares (*Lepus europaea*), 100 rabbits (*Orrctolagus* spp.), 5 water voles (*Arvicola amphibis*) 99 common shrews (*Sorex* spp), 7 moles (*Talpa europaea*), 7 hegdehogs (*Erinaceus europaea*), 1 stoat (*Mustela enterica*), 1 weasel (*Mustelanivalis*), 2 badgers (*Metes meles*) and 6 foxes (*Vulpes vulpes*) were examined for *Salmonella* (Jones and Twigg, 1976). The tissues examined were liver, spleen, intestines and in some cases rectum and mesenteric lymph nodes. One *S.* Typhimurium and 7 *S.* Dublin were isolated from only house mice (Jones and Twigg, 1976).

Guilbdde et al. (1962) isolated five *Salmonella* serotypes from intestinal contents of one hundred and forty nine samples of hippopotamus (*Hippopotamus araphibias)* from East Africa.

Taylor (1968) reported *Salmonella* species isolated from various wild animals including baboon, elephant, giraffe, hippopotamus, monkey and rhinoceros from Kenya, Tanzania and Uganda.

In another study, Gitter and Brand (1969) examined faecal samples from 22 species of wild animals and birds in the Nairobi National Park and 42 species from wild animals in the orphanage. Five samples from the park yielded *Salmonella*: two from hyena, one each from Kongoni harte-beeste (*Alcelaphus buselaphus cokei*), giraffe (*Giraffa reticulata*) and ostrich (*Struthio camelus*).

Salmonella infections in the Indian elephants (*Elephas maximus*), African elephants (*Loxodonta africana*) and black rhinoceros (*Diceros bicomis*) have been recorded in many parts of the world (Zwart, 1962; Windsor and Ashford, 1972).

Windsor and Ashford (1972) reported the death of two African elephants (*Loxodonta africana*) and one black rhinoceros (*Diceros bicornis*) at the Nairobi Game Park, Kenya which was confirmed to be due to *S.* Enteriditis and *S.* Typhimurium. They concluded that game animals rarely suffer from clinical salmonellosis in the wild and that the disease is more of capture, captivity and contact with man.

Gopee et al. (2000) in the retrospective and longitudinal study of salmonellosis in captive wildlife at the Emperor Valley Zoo in Trinidad and Tobago from 1993 to 1996 reported isolation of *Salmonella* from 17 out of the 141 sick or dead animals. This is a recovery rate of about 12%, with *S.* Typhimurium being the predominant serotype. They reported that 66 out of the 1,012 samples from apparently healthy animals yielded 24 serotypes of *Salmonella*. Sixteen were identified as *S.* Seigburg, six as *S.* Gaminara and six as *S.* Thompson. The prevalence of asymptomatic infections by *Salmonella* spp. in zoo animals was reported to be high.

Falade and Durojaiye (1976) reported that there was no published report on *Salmonella* in wild animals in Nigeria. They noted a few unpublished *Salmonella* isolation from the University of Ibadan Zoo; these included the isolation of *S.* Aba and *S.* Takoradi from a lizard and dead baby elephants respectively by Ojo, Olufemi and Etukudo who also isolated *S.* Typhimurium from a healthy monkey and gorilla from the zoo.

Records of unexplained diarrhoea among captive animals at the Agodi Gardens from the State Veterinary Investigation Laboratory in Ibadan, South west Nigeria prompted a bacteriological survey of wild animals both at the Agodi Gardens and the University of Ibadan Zoo.

Eight *Salmonella* isolates were obtained which are of six different serotypes; six were isolated from the 20 wild animals sampled at the Agodi Gardens, while two were isolated from the 21 wild animals sampled at the University of Ibadan Zoo (Falade and Durojaiye, 1976).

The animals from which *Salmonella* were isolated included putty nosed monkey (*Cercopithecus nictitans*); *S*. Weybridge, Green monkey (*Cercopithecus aethiops*); *S*. Weybridge, patas monkey (*Erythrocebus patas*); *S.* Offa, baboon (*Papio anubis*); *S*. Saint-paul, marsh mongoose (*Atilax paludinosus*); *S*. Saint-paul, civet cat (*Vivera civetta*); *S.* Glostrup, hyena (*Crocuta crocuta*); *S*. Wimborne and *S*. Dublin and Aviary (*Sagitarius serpentarius*); *S*. Wimborne (Falade and Durojaiye, 1976).

Okoh and Onazi (1980) reported the isolation of 14 strains of *Salmonella* from a variety of morbid wild animals, carcasses, and faecal samples of captive wild animals and birds from the Kano State Zoo, Northern Nigeria. The animals and the respective samples that yielded *Salmonella* isolates were pigeon liver; *S*. Typhimurium, parrot small intestine; *S*. Give, peacock liver; *S*. Gallinarum, flamingo faeces; *S*. Apeyeme, pelican small intestine; *S*. Tilene, vulture liver; *S*. Gallinarum, gazelle small intestine; *S*. Epicrates, giraffe liver; *S.* Dublin, galago bush baby small intestine; *S*. Durban, kangaroo liver; *S*. Vejle, hyena faeces; *S*. Oranienburg, cheetah faeces; *S*. Chandans, cheetah faeces; *S*. Rissen, lion faeces; *S*. Vejle, chimpanzee feces; *S.* Liverpool, chimpanzee faeces; and *S*. Elizabethville.

Salmonella Pullorum was reported to have been isolated from the lung, liver, kidney and intestine of a captive chimpanzee (*Pan troglydytes*) at the Jos Zoo, Nigeria by Ocholi et al. (1987). The one year old female chimpanzee was reported to be ill with constant diarrhoea and anorexia and died after five days.

Recently, *Salmonella* was isolated from avian wildlife in the United States of America (Aaron and Emi, 2008). Lecis et al. (2011) isolated *Salmonella* from European tortoises (*Testudo hermanni, Testudo graeca and Testudo marginata*), while in Spain *Salmonella* isolations were made from pet turtles (*Trachemys scripta troosti*) (Lafuente et al., 2013). However, *Salmonella* was also isolated from boars (*Sus scrofa*) hunted in northern Italy.

CONCLUSION

The epidemiology of salmonellosis is complex, involving *Salmonella*, wildlife, livestock, man and the environment. The morbidity and mortality arising from the zoonotic infection is of great concern and high economic value.

The knowledge of the genus and methods of isolation are therefore of great importance. The roles played by wildlife in the epidemiology of the disease may not be fully known and understood. This calls for further research work.

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