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A Brief Account of the Rules Applied to the Naming and Epidemiologically Grouping *Salmonella* Strains when Isolated from Animals

[1]Grammato Evangelopoulou, [3]Spyridon Kritas,
[2]Alexander Govaris and [1]Angeliki R. Burriel

Salmonella spp., the most pathogenic genus of the family of Enterobacteriaceae for man and animals, has many of its pathogenicity determinants still unknown, although it is systematically studied for more than 100 years. This is mainly due to the slow development of methods reliably associating the molecular characteristics of strains or clonal lineages with their observed pathogenicity and epidemiology. The same has hampered the effective control of animal salmonelloses, thus prevention of human infections. However, in recent years, many new molecular methods are developed to genetically, thus also taxonomically, define *Salmonella* spp. and are also useful in better understanding the pathogenicity of the microorganism. A better understanding of the microbe's pathogenicity is the key to the development of effective means, such as vaccines, for controlling animal salmonelloses, regardless of animal species. However, due to their costs and limited molecular information, serotyping, the classical method for many decades of placing *Salmonella* isolates into similar antigenic groups, remains the tool for epidemiologically studying the microorganism, during the surveillance of animal salmonelloses. Serotyping, known as the White-Kauffmann-Le Minor, scheme, has produced during the years a bulk of information contributing to conflicting opinions concerning the nomenclature and taxonomy of the genus *Salmonella*, thus needing constant revision of the rules managing it. Molecular methods are expected to steadily resolve these conflicts but they are yet far from replacing the existing system of naming and grouping *Salmonella* isolates. Thus, a concise summary of the existing scientific opinions and rules influencing still today the grouping of the genus *Salmonella*, could be useful to veterinarians and others working with the surveillance of animal salmonelloses.

Key words: Nomenclature, *Salmonella*, taxonomy, animal, salmonellosis

[1]Laboratory of Microbiology and Parasitology,
[2]Laboratory of Hygiene of Foods of Animal Origin, Faculty of Veterinary Medicine, School of Health Science, University of Thessaly, Trikalon 224, Karditsa, 43100, Greece
[3]Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124, Thessaloniki, Macedonia, Greece

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For further information about this article or if you need reprints, please contact:

Grammato Evangelopoulou
Laboratory of Microbiology and Parasitology,
Faculty of Veterinary Medicine,
School of Health Science,
University of Thessaly,
Trikalon 224,
43100, Karditsa, Greece

Tel: +30-2441066088
Fax: +30-2441066088

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INTRODUCTION

A number of *Salmonella* serovars and strains causing in man typhoid and paratyphoid fever, cause in animals from subclinical infection to severe clinical enteric disease (Acheson and Keusch, 1997). Human salmonellosis is a food borne infection. Thus, infected food producing animals are a public health hazard needing continuous surveillance (SANCO, 2009). Successful surveillance and control of animal salmonelloses depend on the method used to reliably associate strains, significant for Public Health, to their animal source. The fast addition of new serovars on the existing long list, as a result of intensified research in animals, is increasing the complexity of the microorganism's epidemiological classification.

In addition, information about *Salmonella* spp. isolated from animals and reported following the older rules of taxonomy may not be successfully associated to currently reported information, if one is not considering the landmark changes of grouping isolates with the classical methods. The amount of existing information deriving from the application of older taxonomic rules, the many versions of "correct opinions" reported by official microbiological societies and new and older methods employed simultaneously, when taxonomically placing *Salmonella* isolates, are some problems faced by health workers attempting to apply the published methodology.

Until recently, common practice was, when placing isolated strains into species, subspecies, subgenera and serovars, to combine several accepted rules (Brenner *et al*., 2000). During the years, *Salmonella* isolates, named in previous decades as species, were later placed into subspecies or subgenera and eventually into antigenic sero-groups, better suited to epidemiological investigations. The latter number today more than 2610 serovars (Guibourdenche *et al*., 2010). The list of serovars becoming longer by the years, did not fully resolve questions on the clinical and epidemiological significance of serovars. Thus, to reliably associate the clinical and epidemiological manifestations of serovars isolated during the surveillance of animal salmonellosis with disease in man or other host animal species, easier, economical and reliable molecular methods are needed. They should, for success in such programs, better match past information with the findings of current *Salmonella* surveillance and control programs around the world, and most importantly, between regions within the same country. This success depends on the effective management of molecular information generated from various sources studying multiple subspecies and serovars as potential pathogens.

When potential pathogens are searched, detection of any number of microbial cells in samples is evidence of infection, enforcing the undertaking of preventive measures. These measures will be most effective in the case of salmonellosis, if they are targeting serovars of increased economic and Public Health importance. This targeting requires precise knowledge of the genetic composition of serovars pathogenic to various animal species and man.

The very large number of serovars recorded is indicative of a similarly large antigenic variation in the *Salmonella* population. These antigenic variations, manifestated in a variety of clinical ways, are encoded on specific nucleotides, therefore easily exploited by PCR. Several PCR-based methods are exploited, targeting specific genes of either the most prevalent or all salmonellae (Arrach *et al*., 2008). Generally, PCR is used as a highly sensitive and specific method for checking the presence of pathogenic bacteria in clinical specimens and is particularly applicable when high sensitivity is required, as in cases of specimens having numbers of a pathogen undetected by culturing (Cohen *et al*., 1993). In addition, antigenic differences between strains, the result of genes and gene alleles diversity, are also molecularly associated with a strain's phenotype. Thus, an expanded and comprehensive PCR molecular database is needed to firstly accurately place unknown *Salmonella* isolates and secondly select the most important molecules coding for pathogenicity (Wise *et al*., 2009). Until such a database is successfully enriched to be effectively used during epidemiological investigations, serotyping, historically proven useful in such investigations, will be the accepted method.

Thus, a brief account of landmark official decisions forming the taxonomic rules could help the clinical veterinarian to better associate current knowledge on serovars with past information.

A BRIEF HISTORY OF THE RULES APPLIED TO TAXONOMY OF *Salmonella* spp.

The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist (Smith, 1894). Salmon and his colleague Theobald Smith, isolated in 1884 from a pig's intestine suffering "*Hog cholera*", a microorganism they assumed it was the cause of the illness. They named it "*Bacillus choleraesuis*". "*Bacillus choleraesuis*" was elevated to the level of a genus in 1900 by the French bacteriologist Liengieres and named "*Salmonella*" (The *Salmonella* Subcommittee of the Nomenclature Committee of the International Society

for Microbiology, 1934). The genus *Salmonella* Liengieres included all known Gram (-) bacteria, among which were "*Bacillus typhimurium*", "*Bacillus typhi*", "*Bacterium paratyphi*", "*Bacillus enteritidis*" and of course "*Bacillus choleraesuis*" (Brown, 1935). As information accumulated the following decades on the genus *Salmonella*, it became evident that a more precise system of taxonomically placing the microorganism was needed. Thus, *Salmonella* isolates were initially named to species according to their clinical manifestations, taking names such as *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis* or named after their host as e.g., *Salmonella gallinarum*, *Salmonella abortusovis*, *Salmonella choleraesuis* or their geographical origins, named as e.g., *Salmonella london*, *Salmonella panama* (The *Salmonella* Subcommittee of the Nomenclature Committee of the International Society for Microbiology, 1934). This complicated system of naming isolates did not epidemiologically associate the various isolates, thus, the antigenic composition of isolates (serotyping) was attempted. This method answered many surveillance questions but it also further complicated the interpretation of existing and new information (Le Minor and Popoff, 1987; Agbaje *et al*., 2011). Serotyping of each strain was developed in the 1920s on the basis of particular O (cell wall) and H (flagellar) antigens (White, 1926) and expanded during the following decades by Kauffmann (1966) eventually resulting in a large number of serovars. Kauffmann (1966) did actually propose to the scientific community of considering each serovar a separate species belonging to the genus *Salmonella*. If his proposition was adopted, the genus should have by the 70s, before its molecular typing, more than 2500 species; a number completely disassociating epidemiological surveillance from animal and human infections. Evidently, the classification of the genus *Salmonella* has greatly evolved over the years and the rules applied today are the result of numerous compromises and DNA-DNA hybridization (Miller and David, 2000; Euzeby, 1999).

DNA-DNA hybridization first used in the 70s showed that the "species" named in past decades and their serovars were so closely related to each other molecularly, that they could be considered as "one species" (Crosa *et al*., 1973). This new knowledge should have immediately changed the methods of reporting findings but for practical and historic reasons, the list of Approved Bacterial Names, published immediately after the molecular typing of the genus, included, not one but five species. They were *S. arizonae*, *S. choleraesuis*, *S. enteritidis*, *S. typhi* and *S. typhimurium* (Skerman *et al*., 1980). In the years after, Comparative Genomic Hybridization (CGH) assays on whole-genome microarrays showed that genomic differences generally correlated well with a serovar's phenotype, although some exceptions exist. Similarities and differences between serovars do not, however, place them into a specific genogroup. Specifically, phenotypically similar serovars may have substantially different genetic content, thus placed into different genogroups.

The above brief account illustrates the conflicts between those making the rules of naming isolates and those studying their pathogenicity phenotypically or molecularly (Crosa *et al*., 1973; Lim *et al*., 2005; Falush *et al*., 2006; Wise *at al*., 2009). More precisely, the conflicts existing to this day, between microbiologists and clinicians. Their conflicts were partially resolved with the publishing of "Judicial Opinion 80" informing them that, after 2005, isolates should be assigned into two species; the type strain "LT2^{T}" previously known as *Salmonella choleraesuis*, now renamed *Salmonella enterica* and *Salmonella bongori* (Euzeby, 1999). However, immediately after the publishing of Opinion 80 and before its application, the Judicial Commission of the International Committee on Systematic Prokaryotes accepted division of the species *S. enterica* into six subspecies (Truper, 2005; Tindall *et al*., 2005). The name of each subspecies was formed by the name of the type species (*S. enterica*) followed by the epithets *arizonae*, *diarizonae*, *enterica*, *hountanae*, *indica* and *salamae*. Concurrently with this division, a third species was included in the approved list of 2005 (Shelobolina *et al*., 2004). This species is today molecularly placed closer to *Escherichia hermanii* (Skerman *et al*., 1989), forcing, perhaps, soon a new ruling and a new list of official names for the genus *Salmonella*. Most importantly, Judicial Opinion 80 did not invalidate the previously published list of *Salmonella* names. Thus, two lists of officially accepted names were combined and are currently used in the taxonomy of the genus; the one used just before 2005 and the other after.

This combined list consists, hence, of nine species which are *S. arizonae*, *S. bongori*, *S. choleraesuis*, *S. diarizonae*, *S. enterica*, *S. enteritidis*, *S. paratyphi*, *S. typhi* and *S. typhimurium* and 14 subspecies (Skerman *et al*., 1989). The subspecies are named either using the historic name for the type species, *S. choleraesuis*, followed by the epithets *arizonae*, *bongori*, *choleraesuis*, *diarizonae*, *houtenae*, *indica*, *salamae* or using the new name of the type species, *S. enterica*, followed by the same as above epithets. Therefore, the researcher is left to choose the rules for placing an isolate. This freedom, however, does not help toward a better understanding of generated epidemiological observations.

Thus, to epidemiologically relate a pathogenic strain isolated from an animal species with disease in man or other animal species, regions or farms, the antigenic serotyping of the White-Kauffmann-Le Minor scheme continues apparently to be the most appropriate (Grimont and Weill, 2007), although molecular typing is fast developing. If serotyping is eventually officially used simultaneously with existing molecular methods, an even more reliable recording of observed clinical manifastations of salmonellosis could be expected in the future.

WHITE-KAUFFMANN-LE MINOR SCHEME

The White-Kauffmann- Le Minor, scheme classifies members of the genus *Salmonella* according to their antigens. The interactions between antibodies and specific surface antigens of *Salmonella* spp. are useful diagnostic and epidemiological tools in many laboratories around the world and correlated well with genomes grouped into genovar clades (Grimont and Weill, 2007).

The White-Kauffmann-Le Minor scheme, divides each subspecies of the genus *Salmonella*, as above mentioned, into serovars, relating effectively epidemiological surveillance and disease outbreak investigations, by characterizing each strain's O (somatic), H (flagellar) and Vi (capsular) antigens (Grimont and Weill, 2007).

"O" Antigens are lipopolysaccharides which are components of the cell wall. There are 67 structurally different O-antigens dividing the genus *Salmonella* into 50 different serogroups, called O-groups. O-antigens are characterized using Arabic numerals: 1, 2, 3...etc., (Grimont and Weill, 2007).

"K" Antigens are subunits of the protein "flagellin" present on strains possessing flagella. Most *Salmonella* serovars express two different H-antigens, helping, perhaps, the microbe to overcome the defense mechanisms of its host. Serovars, such as Typhimurium and Choleraesuis, are expressing both H-antigens, thus they are called "diphasic". Others, such as *Salmonella enterica* ser. Enteritidis and Typhi, expressing a single flagellin type, are called "monophasic". Thus, serovars are placed into two groups called Phase 1 and Phase 2. Antigens of the Phase 1 group are characterized by lowercase Roman letters from "a to z" and those of Phase 2 in Arabic numerals: 1, 2, 3...etc. The non-motile serovars Gallinarum and Pullorum are lacking flagellas, thus, they do not have H-antigens (May and Goodner, 1927; CDC, 2007).

Eventually, each *Salmonella* serovar is identified by a unique combination of antigens named in the following order: Name of subspecies [space] definition of O-antigen [colon] definition of Vi-antigen, if present, [colon] definition of phase 1 H-antigens [colon] definition of phase 2 H-antigens. Between them there is a number of individual antigens separated by commas while the main antigens are separated by a colon. In addition, one should remember, that although the terms "serotype" and "serovar" are equally used when characterizing isolates, the term "serovar" is preferred in the revised "Rules of the Bacteriological Code" (Popoff *et al*., 2004).

Evidently, the White-Kauffmann-Le Minor scheme helps in the grouping of all known antigenic types of *Salmonella* serovars (Popoff and Le Minor, 2001; Grimont and Weill, 2007); becoming an effective and economic epidemiological tool for animal salmonelloses. However genovars do not always match serogroups and serovars placed in the same serogroup may molecularly be placed into a different molecular clade. This, perhaps, is the result of laterally transferred genes into different genovars (Porwollik *et al*., 2004).

A combination of the above rules is currently used by the Centers of Disease Control (CDC) in the USA.

TAXONOMIC SYSTEM OF THE GENUS *Salmonella* USED BY THE CDC

The current taxonomic system used by the CDC recognizes two species, *S. enterica*, *S. bongori* and six subspecies within the species of *S. enterica*. The subspecies are *S. enterica* subsp. *enterica*, also known as subspecies I, *S. enterica* subsp. *salamae* or subspecies II, *S. enterica* subsp. *arizonae* or subspecies IIIa, *S. enterica* subsp. *diarizonae* or subspecies IIIb, *S. enterica* subsp. *houtanae* or subspecies IV and *S. enterica* subsp. *indica* or subspecies VI (Su and Chiu, 2007). Today, most serovars molecularly typed are belonging to *S. enterica* subspecies I (99.9%) and few to subspecies II and IIIb. Thus, the large number of serovars in subspecies I is requiring the proper naming of serovars within it for avoiding confusion during the matching of epidemiological investigations from around the world. For this purpose, two methods of reporting information on serovars are internationally accepted. The one previously explained and the one preserving to this day historic names. The latter, however, used the rules applied to naming species, long after the molecular typing of serovars (Grimont and Weill, 2007), thus confusing many researchers or clinicians thinking them as species. For avoiding such a confusion, the rules of naming historic serovars (previously known as species) changed and they are now reported strictly following the order: Italicized name of the species and subspecies, e.g., *Salmonella enterica* subsp. *enterica* followed by the

non-italicized abbreviated word ser. for "serovar" and this followed by the capitalized but not italicized second synthetic of the name of a historic previous species, now molecularly considered a serovar (Agbaje *et al*., 2011). Eventually the name of a historic serovar is reported as in e.g., *Salmonella enterica* subsp. *enterica* ser. Typhi. The name may be shortened in later mentions using only the italicized name of the genus (*Salmonella*) and the non-italicized, capitalized name of the serovar, as in e.g., *Salmonella* Typhi. In the last case, the genus cannot be abbreviated as in S. Typhi, a practice used when reporting names of species. However, permitted is the use of only the non-italicized but capitalized last synthetic, as in "serovar Typhi" (Grimont and Weill, 2007). Serovars belonging to the other subspecies of the type species, mainly associated with the cold-blooded animals (De Lappe, 2009), are reported according to their antigenic composition following the rules explained previously. An example is, serovar *Salmonella enterica* subsp. *salamae* 39:z10:z6 or serovar *Salmonella* II 39:z10:z6.

Worth mentioning here is that for a brief time in the past, the genus *Salmonella* was also divided into subgenera (Le Minor *et al.*, 1970). According to this division, *S. bong*ori, molecularly defined and accepted as species after 1973, was before this typing, a member of subgenus V. Thus, serovars of the species *S. bongori*, previously placed in the subgenus V, continue to keep the roman letter V and they are written as serovar *Salmonella bongori* V 13, 22:z35: or *Salmonella* V 13, 22:z35: (Popoff *et al*., 2004).

Responsible for the revision of the White-Kauffmann-Le Minor scheme is the Pasteur Institute in Paris which is the WHO's Collaborating Centre for Reference and Research on *Salmonella*. Another critical centre for recommending rules and changes concerning the genus *Salmonella* is the CDC and both taking into account information from molecular methods used around the world.

CONCLUSION

The many scientific opinions published on the rules of naming and epidemiologically grouping *Salmonella* strains through the years have hampered, perhaps, at times the successful attempts of epidemiologically investigating, thus effectively controlling, animal salmonelloses. The bulk of information produced to this day by the biochemical, molecular or epidemiological methods used to classify the genus is complex and ultimately confusing, to those attempting to associate a serovar with an animal host-species (Agbaje *et al*., 2011). One should remember, when studying *Salmonella* infections, that some clinicians (medical doctors and veterinarians) may still report their clinical findings using older taxonomic rules, thus confusing their younger colleagues familiar with newer methods, such as molecular.

On the other hand, although molecular information is fast accumulated, Opinion 80, a "consensus" between clinicians and taxonomists, hasn't yet been fully adopted by organizations, such as the CDC or the WHO's Collaborating Centre (Grimont and Weill, 2007). This slow adoption by renowned laboratories of officially set rules for such an important microorganism, illustrates the difficulties encountered when the new must successfully merge with the older. These difficulties negatively influence also the application and final acceptance of newly developed and developing molecular methods. It appears, therefore, that there is a long way before molecular methods replace the older rules applied to the epidemiological grouping of *Salmonella* isolates.

The current List of Approved Names which is a compromise between all those methodically studying the microorganism for many decades, is also the link between old and new information concerning this important pathogen causing today the majority of food born illness around the world. Increased access to molecular methods around the world for epidemiologically characterizing isolates of the genus *Salmonella*, need to take into account the above to successfully replace serotyping, helping consequently the better understanding of this microorganism's pathogenicity, thus the effective control of animal infections.

Leaders in these changes for a guaranteed success should be organizations and laboratories, such as the WHO and the CDC having great experience in properly placing *Salmonella* isolates but also money to further develop new molecular methods and computerized data libraries. Until then, the bulk of information reported by scientists studying human and animal salmonelloses will continue to add difficulties in defining the pathogenic importance of newly isolated strains and, most importantly, studying the adaptation of serovars to new animal hosts.

Serotyping scores in the third External Quality Assurance of *Salmonella* typing (EQA) were found acceptable, due to that 90% of all strains were correctly serotyped. However, in regard to participating laboratories only 15 of 26 (58%) correctly identified all serovars. One EU laboratory identified only 20% of the serovars correctly while another misclassified some of the most common serovars (Pol-Hofstad *et al*., 2012). Thus,

with an accepted statistical threshold for correct results put at 90, 81% of laboratories would pass. However, in such a case, considering that about 100000 cases of salmonellosis are reported annually to the European Centre for Disease Prevention and Control (ECDC), about 10000 cases would be reported as caused by the wrong serovar and unknown is the number of false negative samples. The problems mainly lie in the typing of H antigens, with subsequent misnaming of the serovars and the limited sensitivity of culturing the microorganism from samples. Perhaps, such problems could be resolved, if a properly chosen molecular method is simultaneously used with culturing and serotyping.

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