Of Salmonellae and *Salmonella* Gozo

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Introduction

I will always remember the summer of 1993 as a particularly hot and humid season, not to mention my experiences chasing, with gloved hands and booted feet, a few hundred apprehensive and distrustful cows for some stinking, fresh dung. The memories now are quite hilarious, but not then, at least not when the bulls or a rather angry cow had to be confronted.

Having always been rather keen on the study of micro-organisms, I knew right from my freshman year at the University of Malta, that I wanted to work in a topic related to microbiology for my undergraduate thesis. In 1991, I spent a whole summer reading Medline abstracts, but it was an article on *Salmonella* in one of my Medicine Digest copies that actually caught my attention. Another reason that encouraged me to undertake a study on *Salmonella* was the fact that no such studies on animals had been carried out before in Malta. I remember writing a brief protocol about what I wanted to do, going to the Bacteriology laboratory in St. Luke's Hospital and asking Dr. PauI Cuschieri, the Consultant Bacteriologist in charge of that laboratory, if he could be my tutor and if what I had in mind was feasible. Happily enough, he was quite interested and accepted without hesitation. So started a long and eventful study on salmonellae. Initially, I wanted to study salmonella carriage, infection and disease in poultry but complications arose, primarily because permission to visit poultry processing plants was almost impossible to obtain. Next, I focused my attention on pigs; however, I practically knew no one in Malta who could take me around the various pig farms in Malta. In the end, keeping in mind the limited time I had to carry out the study, I realized that Gozo was just small enough and had just the right number of cattle farms for my project. There were other advantages: my father could help to take me around the various farms for the samples (since I had no car) and I could speak the various Gozitan dialects, which made the farmers more receptive.

The Genus *Salmonella*

Salmonella is a group of bacteria consisting of more than 2000 different types (known as serotypes or serovars); these are all potentially pathogenic to man and may cause gastroenteritis, septicaemia or enteric fever. Salmonellae also infect many animal species, including birds and reptiles. In man, infections often result following ingestion of improperly cooked animal products that have been previously contaminated with faecal matter during processing. This results in an acute, self-limiting gastroenteritis[1] or a systemic infection (enteric fever) involving other areas of the body. Patients with acute gastroenteritis excrete large numbers of bacteria in faeces but numbers diminish as recovery progresses, so that after 3 to 4 weeks, stools are usually bacteria free. However, some patients continue to excrete salmonellae for a longer period, which ranges from months to several years, although permanent chronic carriage lasting for more than one year is very rare. The case fatality rate is low, usually less than 0.4% and deaths mainly occur in the very young, the very old and in debilitated, immuno-compromised persons.

The generic term *Salmonella* was given to the micro-organism in 1900 in honor of Dr. D. E. Salmon (1850-1914), an American pathologist who, together with Theobald Smith, was the first to discover and describe *Salmonella*. On the surface of this bacterium, there are various proteins called antigens, which differ in their nature between the different salmonellae. This enables scientists to distinguish between one *Salmonella* serovar and another; thus, *Salmonella*

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[1] The gastrointestinal form is often referred to as food poisoning, although this is a misnomer, since the disease is an infection rather than an intoxication.

Enteritidis has different antigens on its surfaces compared to say, *Salmonella* Montevideo. Historically, salmonellae have been classified according to their antigenic structure (i.e. the types of antigens present) and each new type that is discovered is named after the place in which it was first isolated. The Kauffmann-White scheme is a systematic tabulation of the different antigenic structures of the *Salmonella* serovars known. In this classification system, the genus *Salmonella* (abbreviated to *S*.) is divided into two species, namely, *S. enterica* and *S. bongori*. *Salmonella enterica* is further subdivided into 6 subspecies, the most important of which is *Salmonella enterica* subspecies *enterica*. Most of the *Salmonella* serovars pathogenic for mammals and birds belong to this subspecies, although members of *S. enterica* subspecies *arizonae* are important causes of diarrhoeal illness in turkeys and are therefore of worldwide economic importance.

The Study

In 1993, I therefore started a cross-sectional study on a representative sample of cows and bulls from various farms in Gozo, the main aims of which were:

1. to determine the prevalence of *Salmonella* excretion/carriage in these animals;
2. to identify the most common serovar prevalent within the study population; and,
3. to determine the presence of any serovar resistance to the antibiotics commonly used in animal husbandry.

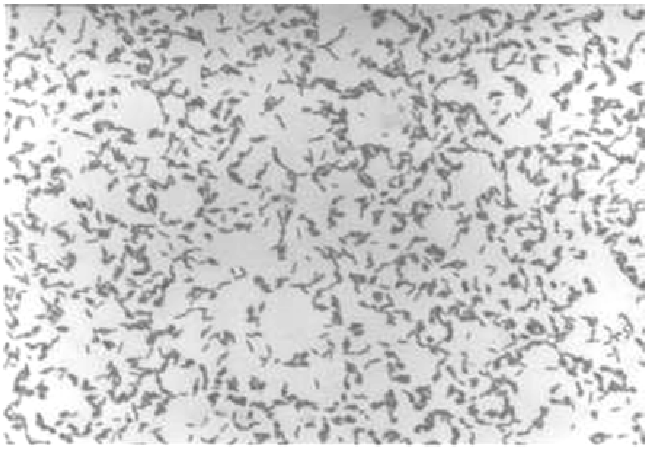
The study population consisted of 2325 cows and 68 bulls. A representative sample of 300 head was selected using strict random sampling from 62 farms spread in 14 towns in Gozo. These consisted of 296 cows and 4 bulls; the animals were from 3 to 4 years old and the cows consisted of both wet (milked) and dry (non milked) animals. Calves and one year old heifers and bulls were excluded.

Faecal swabs were obtained every week for nine consecutive weeks from each individual animal chosen from 52 farms distributed in 12 towns in Gozo. The 300 bovines sampled represented 12.5% of the total bovine population of Gozo whilst the 52 farms sampled constituted 83.9% of the total available sampling points (farms = 62). Each swab was placed in a special medium to protect it, stored at 4ºC whilst in transit and processed in the laboratory within 24 hours of collection. Isolation and identification of the *Salmonella* isolates were carried out using standard procedures. Antibiotic sensitivity testing on the isolated strains was performed using the impregnated disc diffusion method (Stokes' modification of the comparative method); the antibiotics chosen included ampicillin, cefuroxime, chloramphenicol, ciprofloxacin, furazolidone, gentamicin, sulphafurazole, tetracycline and trimethoprim. Throughout the study adequate internal quality control was carried out including quality control on culture media used for the isolation of *Salmonella* serovars, quality control during the actual isolation and identification of the organisms, controls during serological testing and controls used for the antibiotic sensitivity testing.

Results and Discussion

Of the 300 faecal samples taken, 41.3% (n = 124) yielded *Salmonella* and more than one serovar were isolated from seven swabs; hence, there were 131 isolates in all, which increased the percentage of *Salmonella* isolated to 43.7%. Since a single faecal swab was taken from each animal, this means that 41.3% of the animals sampled were excreting the organism at the time of sampling with 7 of the animals excreting more than one type of *Salmonella*.

The cows and bulls yielding positive *Salmonella* cultures did not show any clinical signs of salmonellosis such as fever, dullness, diminished appetite, blood-stained faeces and in the case of cows, reduced milk yields. Since the animals were excreting the organism, it is more likely that they were *Salmonella* carriers rather than cases of acute infection. However, from this study, it cannot be concluded whether this was temporary or permanent carriage. The nature of carriage is particularly important because it affects the persistence of *Salmonella* in the herds. Additionally, cattle from Gozo are used for human consumption both in Malta and Gozo. This increases the opportunity of *Salmonella* dissemination at the abattoir prior to slaughter with a consequent increase in the risk of meat contamination.



Grum stain of Salmonella showing Grum-negative rods (x1000)

Seven different kinds of serovars were isolated, including a new serovar for which the name *Salmonella* Gozo was given (Table 1). When taken together, the three serovars *S*. Croft, *S*. Telaviv and *S*. Montevideo, constituted up to 33.7% of the total number of samples taken. Thus approximately one in every three bovines sampled might have been carrying one of these three serovars. The relative proportions of the serovars isolated may affect the likelihood of the infection of persons or other animals; this is also influenced by the pathogenicity of individual *Salmonella* serovars. In several countries, *S*. Dublin and *S*. Typhimurium are the most common and most pathogenic serovars causing serious clinical infection in adult cattle and calves.

However, at the time of the study, this does not seem to be the case in Gozo, because neither serovar was isolated. Also, since the main *Salmonella* serovars isolated from man locally are *S*. Enteritidis and *S*. Typhimurium, (which were not isolated from any of the animals sampled), it is very probable that *Salmonella* serovars in beef are not a likely cause of *Salmonella* infections in Gozo. This could be due to very efficient cooking of meat dishes. However, lack of reporting of individual cases especially if mild or no symptoms are experienced have to be kept in mind. It could also be due to the fact that the serovars isolated from the cattle studied have a low pathogenicity and are easily destroyed by proper cooking.

***Salmonella* serotype**	**Antigenic structure**	No. of Isolates
Croft	28: g,m,s: e,n,z_{15}	55 (18.3%)
Telaviv	28: y: e,n,z_{15}	29 (9.7%)
Montevideo	6,7: g,m,s: 1,2,7	17 (5.7%)
Gozo	28: e,h: e,n,z_{15}	14 (4.7%)
Kpeme	28: e,h: 1,7	7 (2.3%)
Infantis	6,7: r: 1,5	2 (0.7%)
Abadina	28: g,m: e,n,z_{15}	1 (0.3%)
Other*	-	6 (2.0%)
Total		**131 (43.7%)**

* These were rough strains that could not be serotyped and hence, identified; consequently, they cannot be classified under any serogroup.

Table 1: ***Number and types of Salmonella serovars***

The New *Salmonella* Serovar

A total of 14 *Salmonella* isolates belonged to a serovar that was subsequently confirmed to be a new serovar by the WHO Collaborating Centre for Reference and Research on *Salmonella* in France. All isolates were biochemically typical of the *Salmonella* genus. This isolate could not be serotyped easily with the commercial antisera available to me and it could not be classified in the then current edition of the Kauffmann-White scheme. I determined that the serovar belonged to the O group 28 but it showed antigens in just one phase, (the e,h antigens), while the antigens in the other phase could not be determined even after repetitive attempts (unfortunately, at that time, the hospital laboratory also experienced a shortage of antisera which are necessary for the identification of each *Salmonella* isolate). I had to send all the isolates to the WHO Centre in France for full identification; it was subsequently reported by this Centre that this serovar had the antigenic structure of 28: e,h: e,n,z_{15}. The fourteen isolates were cultured from specimens taken from farms in Nadur (n = 1; one farm) followed by Sannat (n = 3; two farms) and Xewkija (n = 10; three farms). Although the first specimen to yield *S*. Gozo was taken from a farm in Nadur (the farms in Nadur were the ones which I visited first in July 1993), it was one of the isolates from Sannat which was first fully confirmed to be a new serovar by the reference laboratory (from my laboratory log-book records which I still have, the date in which I took the sample that yielded this particular isolate was 2 August , 1993). The ten isolates of *S*. Gozo from Xewkija were obtained from three different farms in this town; direct or indirect contact between the animals on these three different farms is probably responsible for the spread of this new serovar.

When news came from France that the serovar was new and needed a name, my first thought was *Salmonella* Gozo but I wanted to discuss the issue with my tutor and ask for his opinion. Dr. P. Cuschieri suggested *Salmonella* Threehills and *Salmonella* Calypso, but I wasn't very happy with this choice. I wanted a simple name and a name which people would associate immediately with a geographically known location. Strictly speaking, the name should have been *Salmonella* Nadur because that was the place from where it was first isolated. Still, Nadur is not as well known as Gozo and so, I still held in favour of the name *S*. Gozo, which, after much good-natured argument, also satisfied my tutor. Hence, the name which we eventually proposed for this new serovar is *S*. *enterica* subspecies *enterica* var. Gozo or briefly, *Salmonella* Gozo. This name was accepted by Prof. Popoff (with whom I was corresponding through Dr. Cuschieri) at the WHO Centre for *Salmonella* in France and is today listed together with the other *Salmonella* serovars in recent issues of the Kauffmann-White scheme.

Apart from the taxonomic and epidemiological significance associated with the discovery of a new serovar, the isolation of such a strain has other implications. Since the bovine population in Gozo is almost completely isolated from other bovines, this serovar may have become adapted to the island due to particular environmental factors or farming practices found only in Gozo. If this holds true, such factors have still to be determined. Further studies are required to reveal how diffuse this serovar is on the island. To my knowledge, no such studies have been conducted yet.

Further Points

As a result of strict random sampling, the number of animals sampled were proportional to the total number of animals in each locality (Table 2). The basic trend appears to be one of higher *Salmonella* incidence in localities situated in the SE and SW regions of Gozo. The seven localities (Fontana, Għajnsielem, Kerċem, Munxar, Nadur, Sannat, Victoria and Xewkija) that have the highest percentage of *Salmonella* positive animals are all geographically adjacent to each other. The other localities are quite remote compared to these seven regions. The close proximity of these localities lends strength to the hypothesis that easy contact between farmers and farm workers in these places, provides ample opportunity for the transfer of *Salmonella* between one farm and another through commonly shared tools, machines and other fomites.

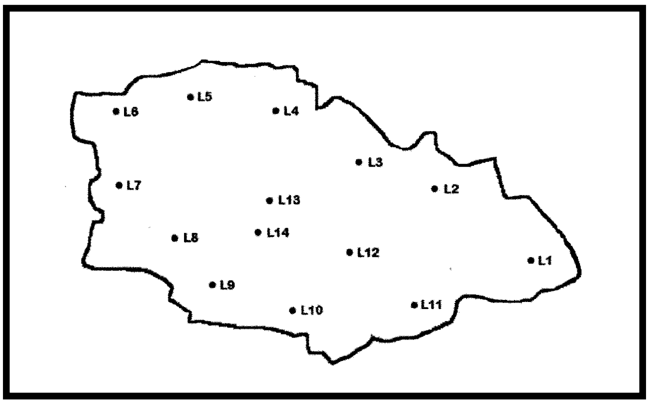
Pigeons and other birds kept on the farms investigated may also be responsible for the carriage of salmonellae from one place to another. These birds are mobile and may defaecate onto, and thus contaminate, water reservoirs and animal feeds. The cycle is complete when the cows become infected and start shedding the organism in the environment thus promoting further infection and colonisation of other animals. Other animals that may contribute to the spread of salmonellae in different farms include dogs, cats and rats. The use of pressure hoses to clean and disinfect animal pens may cause aerosols to form and spread salmonellae to other parts of the premises and thus further contributes to the spread of infection amongst animals. Transmission of salmonellae may also occur during breeding; in Gozo, bulls are sold or transferred from one farm to another for mating purposes.

Town	No. of bovines per locality	No. of animals sampled	No. of *Salmonella* Isolates
Xewkija	629	104	44
Victoria	510	51	22
Kerċem	384	36	16
Sannat	359	49	32
Għarb	124	13	1
Nadur	101	15	7
Munxar	92	9	5
Xagħra	62	9	0
Għajnsielem	44	5	3
Fontana	41	5	1
San Lawrenz	34	2	0
Żebbuġ	13	2	0
Total	**2393**	**300**	**131**

Table 2: ***Percentage frequency of isolates per town***

No salmonellae were isolated from San Lawrenz, Xagħra and Żebbug, which are relatively further north compared to other localities. There is no clear explanation for this result. Admittedly, the number of animals sampled from these regions was small compared to the other localities, but then, the total bovine population in these areas is also small compared to that of other towns.

It is common practice in Gozo to house all bovines in a large barn or else divide the herd into small numbers and house them together in smaller pens. Herding the animals together facilitates transfer of salmonellae from one cow to another. After milking, during the day, the animals are almost always allowed to stroll freely in a large open yard and this may enhance transmission, apart from the possible contamination f feeds in troughs. In Xagħra and San Lawrenz, the bovines were kept in sheds of 6 animals each. This partial isolation may limit transmission of infection from one animal to another.



LEGEND

L1	Qala	**L8**	Kerċem
L2	Nadur	**L9**	Munxar
L3	Xagħra	**L10**	Sannat
L4	Żebbuġ	**L11**	Għajnsielem
L5	Għasri	**L12**	Xewkija
L6	Għarb	**L13**	Victoria
L7	San Lawrenz	**L14**	Fontana

Antibiotic Sensitivity Tests

All the antibiotics used in this study have applications in both human and veterinary medicine with the exception of furazolidone which is used exclusively in animals. At the time of the study, the farmers reported using the following antibiotics to treat infections in their cattle: penicillins, oxytetracycline, streptomycin, trimethoprim with sulphamethazine and gentamicin. However, they did not mention using chloramphenicol, cefuroxime, ciprofloxacin and furazolidone.

During testing, the strains gave definite results, there were either sensitive or resistant - practically no strains showed intermediate resistance. The majority of strains were sensitive to most of the antibiotics used but most strains (n = 98, 74.8%) of all serovars (excluding *S*. Abadina), were resistant to sulphafurazole. Resistance to sulphonamides by *S*. Montevideo was found to be greater than with the other serovars.

Despite the widespread use of penicillins and oxytetracycline for the treatment of infections in the bovines studied, relatively little resistance was found to these antibiotics. It was most reassuring that no resistance to the newer antibiotics (ciprofloxacin and cefuroxime) was observed.

Conclusions

By March 1994, I had completely finished my study and writing it up. I remember then being completely immersed into studying for the looming final examinations and so, I was greatly surprised by the publicity which followed when the Head of the Pharmacy Department broke the news of the *S*. Gozo isolation in a press conference, because I was not expecting it. However, when I look back, it is the lesson of being prepared and of perseverance in doing one's work right the first time that comes instantly to mind. Quoting the great Louis Pasteur, "*Chance favours the prepared mind*", when one prepares well and meticulously carries out the task at hand from beginning to end, no matter how laborious the work or what the subject matter is, the end result is always worth the efforts put in. Finally, I have always believed that what one starts, one must finish and then, one has to move on.

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In the 19th and 20th century, Gozitan ladies produced extremely beautiful masterpieces especially church vestments with these motifs. This inspired me to create my project for the Certificate in Lace Studies course. I selected four types of '*festuni*' and made a lace insertion for a small curtain to cover a cupboard's glass pane. I used linen cloth and linen thread as linen is a very durable type of material.

Linen is one of the earliest products known to mankind. It was widely used by the Egyptians and other civilizations in later periods. Linen is made from a plant know as flux and its main areas of cultivation are Russia, Belgium, France and the Netherlands. There is also proof that flux was cultivated and linen was produced in the Maltese Islands. In fact, in the valley between Sannat and Xewkija in Gozo there are baths, which are believed, were used for the manufacture of linen.

'Balla' Lace and the Armorial Altar Lace Insertion at Casa Rocca Piccola, Valletta.

ANNA MARIA GATT

Maltese '*Balla*' lace incorporating liturgical symbols, feature predominantly in church vestments such as in lace trimming for altars. Many lace workers worked and contributed lace for ecclesiastical purposes. My research features the historic and cultural events leading to the introduction of the '*Balla*' lace in the Maltese Islands and how Church lace became characterised as '*tal-Balla*'. The technical drawing of the '*Balla*' stitch, its technique applied in Maltese lace and its significance, are also discussed and illustrated.

Cathedrals, churches and museums have extensive collections of altarpieces. One such altar lace edging design depicting an armorial motif is in the private collection of Marquis Nicholas de Piro at Casa Rocca Piccola, Valletta. The history, origin, design and commissioning of this particular lace work are also discussed in this research.

The reproduction of a section of the original pattern, the use of old thread and technical drawing of the boll stitch and other techniques used in Maltese lace explaining the sequence of work, are all included in this project.

(Continued from page 16)

Acknowledgments

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