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**A Study on the Occurrence of Salmonella in
Maltese Hunting Dogs**

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1. Introduction

The ownership of dogs for hunting and scavenging purposes in Malta is a common occurrence, the present study was initiated to ascertain the incidence of *Salmonella* shedding in the respective dogs. The dogs included in this study were of 2 main categories:

- Hounds which are kept solely to hunt for wild game for food consumption or for sport.
- Canines residing on a variety of farms for the sole purpose of pest control.

Wild game hunting dogs:

Located in the centre of the Mediterranean Sea between Europe and Africa, Malta has always relied on bird-migration for the hunting of wild birds using the island as a stop-over. This migration route happens twice a year - in spring and autumn. Hunting is permitted for 40 species from land and 12 species hunted at sea. However, the Turtle Dove and Quail are the main quarry of Maltese hunters. Another less popular practice is the Wild Rabbit hunting which usually occurs from June till December every year. These type of hunting practices on the island are recognised under the laws which take into account cultural, economic and recreational requirements.

In order to aid in the retrieval and hunting of wild game and rabbit, most hunters are usually accompanied by their canine companions (Figures 1 and 2). The most popular dogs chosen for these hunting practices are mainly the “Kelb tal-Fenek” also known as the Pharaoh hounds which are appreciated by Maltese farmers for their exceptional ability to hunt rabbit, together with the “Kelb Tal-Kaċċa” translating into “the hunting dog” due to



Figure 1

Dogs announcing a hidden prey.

Credits: <http://www.kelb-tal-fenek.com/action.htm>



Figure 2

*Old photo of a hunter
accompanied by his hunting dog.*

Credits: <http://www.kelb-tal-fenek.com/action.htm>

their ability for retrieval of hunted wild game. Nonetheless, a number of other dog breeds are equally chosen to accompany hunters with their hobby.

Farm dogs used for pest-control:

Another important category of dogs tested within this study include those dogs which are inhabiting many farms containing a number of different food-producing animal species (Figure 3). A variation of dog breeds are usually chosen for this purpose however the ones commonly chosen in Malta include any terrier-type dog specifically Smooth fox-terriers together with German pointers, Kelb tal- Fenek and Kelb Tal-Kaċċa. These dogs are usually kept for the sole purpose of hunting wild rodents and scavenging any unwanted new-comers within the farms.



Figure 3

Terrier-type dogs hunting a rat.

Credits: <http://www.deckerhuntingterrierregistry.com/newsletter/april2010/temperament.html>

Enteric pathogens including *Salmonella*, may be more frequent in dogs which are highly exposed to the outdoors and contaminated environments due to the exhibition of scavenging and predatory behaviours. Furthermore stress linked with outdoor life (including farm dogs) could increase the probability for infection with *Salmonella* or other pathogens. For this reason, hunting and scavenging dogs have the ability to transmit a number of pathogens into the surroundings including to other animals and human beings (Leahy et al., 2016).

Essentially the clinical signs and symptoms of salmonellosis in warm blooded vertebrates are the same, these include: Diarrhoea, vomiting, nausea, depression, fever, headache, muscular pain and general weakness. The most important signs being: Severe dehydration due to fluid loss and electrolyte imbalance which in severe cases could lead to circulatory collapse, convulsions and eventually death (Morse et al.,1976). In spite of this, it must be noted that most *Salmonella*-infected dogs do not show any clinical signs and are completely asymptomatic when being harboured by this pathogen, while simultaneously intermittently shedding the bacteria (Morse et al., 1976).

This study was initiated as a first step towards the analysis of the extent of *Salmonella* incidence within the Maltese hunting and scavenging dog population. This study may also be useful to spread awareness towards the risk of these companion animals transmitting *Salmonella* to other dogs, to any surrounding animals they come in contact with and especially the human health significance due to the intimate relationship between “man and his best friend”.

2 Literature review

2.1 Prevalence of *Salmonella* in dogs and its route of infection

Salmonella is the aetiological agent of salmonellosis and it is one of the most important zoonotic diseases with a global distribution. Studies of different canine populations worldwide have shown that the appearance of *Salmonella* strains is not a rarity in dogs (Morse et al., 1976). *Salmonella* species are Gram-negative facultative anaerobes causing various forms of disease.

The Maltese islands inhabit around 12,161 hunters presently (2.6% of the total population) which happen to own one or multiple hunting dogs to assist them in their hobby (The Malta Independent, 2019). In addition to hunting, these dogs are usually family dogs and so they are frequently kept in close proximity to humans. Since dogs have a tendency to scavenge and be coprophagic, there are plenty of opportunities for transmission and reinfection with *Salmonella* species.

The majority of dogs infected with *Salmonella* rarely show any signs of infection, if so the symptoms are usually mild, therefore making it hard for detection. The dog is second to the pig on the rank of prevalence of salmonellosis and the number of serotypes harboured according to Moran (Morse et al., 1976). A number of surveys conducted worldwide approve the fact that salmonellosis in dogs is not a scarce finding, in fact around 10% of dogs probably encounter infection during their lifetime (Morse et al., 1976).

As stated before salmonellosis in dogs may be asymptomatic while the agent can be intermittently shed. The route of infection in dogs is per os via food, water or fomites (Schotte et al., 2007; Finley et al., 2008). Infection can spread through direct contact with an infected animal and between animals and humans. The largest cause of spread is primarily through faeces of diseased individuals via the oral route (The Swedish National Veterinary Institute, 2004). Leonard et al. (2011) found that the dogs were more likely to be confirmed positive for salmonellosis when:

- in contact with livestock.
- were treated with probiotics in the previous 30 days.
- were fed raw foods likes raw meat and raw eggs.
- fed a commercial or homemade raw food diet.

- fed a homemade cooked diet.
- having more than one dog inhabiting the house.

Prevalence of *Salmonella* was also slightly varied between season in accordance to the study by Stucker et al., (1952) where greyhounds seemed to be more likely to be positive during the late fall and early winter period, while having lower rates of infection during late winter, spring and summer. Bagcigil et al., (2007) also suggested that stress, treatment of dogs with antibiotics, transportation and diets including raw meat may increase the risk of salmonellosis.

Various studies on the successful isolation of *Salmonella* serotypes in dogs around the world have differing results. Table 1 shows some of the *Salmonella* isolation rates in various geographical locations.

From 100 rectal swabs taken from dogs showing no clinical signs in Tehran in Iran, 22% of dogs were positive for *Salmonella*. It is thought that contaminated foods may be the cause of infection in the population tested (Hashemi et al., 2016).

In contrast to low percentages in most of the studies done, Cantor et al. (1997) found a very high prevalence in Alaskan sled dogs (63%). Differences in the results may be due to the different geographical locations, different sampling strategies and methods of conducting the experiments. Other factors which may lead to some studies having higher/lower results than others are differing living conditions, lower stress factors, good quality feed, different sanitation procedures and sample timings (Bagcigil et al., 2007; Cantor et al., 1997).

In a study conducted in North-central Nigeria, the researchers suggest that there are several factors affecting the ease of infection within a canine population, one of these factors being age. In fact, there was a lower incidence rate of infection in dogs which were 4 years or younger within their study. Researchers suggest that this could be due to the maternal antibodies present in dogs within this age bracket, providing natural protection from infection. In contrast, older dogs are more prone to be immunosuppressed resulting in a higher probability of incidence. Another factor discussed in this study was the breeds of dogs being tested. Interestingly, researchers encountered a higher prevalence of *Salmonella* infection in exotic breeds of dogs (having well-off owners) rather than Nigerian local breeds (Mongrels) which were mostly kept by the poor people within the community.

Geographical location	Prevalance rate (%)
New Zealand	0
Slovakia	0.53
Zaria	1.0
Nigeria	1.0
Istanbul, Turkey	1.0
Eastern Washington	1.2
South-Western Ontario, Canada	1.2
North-Central Colorado	2.3
Italy	2.4
Northern Bavaria, Germany	3.45
Trinidad	3.6
Taiwan	4.3
Texas	4.9
Makrudi, Benue State, North-Cental Nigeria	5.5
Japan	5.9
Northern Taiwan	6.3
Brisbane area	6.9
Bursa, Turkey	11
USA (racing greyhounds)	11
Honolulu, Hawaii	11.2
North-Eastern Thailand	12.4
Florida	15
Tehran, Iran	22
USA (racing greyhounds)	44
Alaska	63

Table 1

Worldwide incidence of Salmonella isolations from canine populations

Sources: Frost et al., 1969; Timbs et al., 1975; Cantor et al., 1997; Seepersadsingh et al., 2004; Bagcigil et al., 2007; Tsai et al., 2007; Procter et al., 2013; Lowden et al., 2015; Hashemi et al., 2016; Leahy et al., 2016; Akwuobu et al., 2018

Although the Nigerian local breed dogs were kept within unhygienic conditions with poor quality food and poor management, they were less likely to be harboured by *Salmonella* (prevalence rate: 2.4%), this is thought to be due to the development and adaptation of a natural resistance to the bacteria. Researchers also stated that the cause of infection in exotic breeds of dogs was a result of the feed they were consuming which was likely to be a commercially prepared *Salmonella* contaminated diet or an unprocessed/raw food diet, particularly meat that was contaminated (Akwuobu et al., 2018). Although Akwuobu et al. (2018) stated that the dogs kept within less than ideal conditions were less likely to be infected by *Salmonella*, “It should be remembered that healthy animals are probably more resistant to salmonellosis than their counterparts with malnutrition, avitaminosis and concurrent maladies, and living under crowded, unsanitary conditions. Again the stressed, traumatized or debilitated individual is most frequent victim of salmonellosis”(Morse et al., 1976).

2.2 Clinical Signs in *Salmonella* Bearing dogs

Several surveys conducted around the world confirm that *Salmonella* is not so infrequent, the lack of awareness could be due to the fact that several infections do not manifest into severe clinical signs in most cases. However, several and variable signs have been identified in some instances when dogs are manifested with salmonellosis. These include: hyperpyrexia (40-41°C), anorexia, diarrhoea, bloody stools, vomiting, weight loss, cough, nasal haemorrhages, hyper-irritability, incoordination, partial posterior paralysis, blindness, “running fits and barking”, depression and lochia. The acute phase of the infection may last from four to ten days and it may develop into chronic intermittent diarrhoea for three or four weeks (Morse et al., 1976).

Pregnant and younger animals seem to be at higher risk to salmonellosis. As a result of this abortion, metritis, stillbirths and death are not a rare occurrence (Morse et al., 1976). The diarrhoea manifested is usually watery or mucoid, though it could also be bloody. In addition to this there was no correlation found between the serotypes and the type of diarrhoea manifested (mild, mucoid, bloody or fluid) (Cantor et al., 1997).

In a study that took place within the UK by Philbey et al. (2013) the major clinical sign reported for dogs affected with salmonellosis was diarrhoea while occasional vomiting

occurred in 7 out of 115 cases. However several other clinical signs and pathologies were shown in a number of dogs harbouring *Salmonella*. *Salmonella* Dublin was isolated from the brain/cerebrospinal fluid of two dogs which developed meningoencephalitis and died after manifesting neurological signs.

S. Typhimurium and *S. Dublin* were each isolated from vaginal swabs or discharges obtained from bitches which had aborted. “*Salmonella* Panama and *S. Montevideo* have been associated with abortion in dogs (Redwood and Bell, 1983), but this bacterium appears to be an uncommon reproductive pathogen in dogs.” (Philbey et al., 2013). *S. Kedougou* was recovered from joint fluid of an 18-month old Irish wolfhound which suffered from septic arthritis. *S. Typhimurium* was also isolated from a five-day-old puppy within a litter which experienced neonatal mortality and which manifested diarrhoea and dysentery before being deceased. Lastly a localised scrotal dermatitis in a 13 year old Jack Russell terrier harboured *S. Enteritidis* Phage type 6, even though it was not thought to be the main cause of the disease as a copious amount of *Streptococcus* species was also recovered from the site of infection (Philbey et al., 2013).

Even though *Salmonella* can be found in the environment, the majority of infected dogs with common species of *Salmonella* (except *S. Typhi*) will harbour and shed the bacteria without any major clinical symptoms, on the contrary the situation may be different if sepsis of the bacteria occurs (Ettinger et al., 2017).

2.3 Diagnostics

The classical method for clinical diagnosis and confirmation of canine salmonellosis is established on the isolation of the organism. The diagnosis is further strengthened when the animal is presenting the typical clinical signs expected when harbouring *Salmonella*, together with the evaluation of the potential risk factors which may have pre-disposed the animal to the disease. These factors include: age, environmental risks, antibiotic administration and previous hospitalisation (Marks and Kather, 2003).

Haematologic irregularities are variable and consist of:

- Non-regenerative anaemia
- Thrombocytopenia
- Lymphopenia

- Neutropenia with a left-shift

Toxic neutrophils are present in canines with systemic disease and endotoxaemia, findings which may be comparable to those documented in canine parvovirus (Marks and Kather, 2003).

Nonetheless, isolation of *Salmonella* does not necessarily signify that it is the main cause of the clinical signs being presented, this is because similar isolation rates may be encountered in clinically healthy dogs (Marks and Kather, 2003).

2.4 Pathogenesis

Distinct mechanisms regarding *Salmonella* pathogenesis have been identified in accordance with the serotype of the pathogen (Sva.se, 2004). For successful infection a large amount of *Salmonella* bacteria are needed due to the lower susceptibility of carnivorous animals. On the contrary, several predisposing factors can increase the likelihood of infection such as cases of immunodeficient dogs or dogs of a young age.

Salmonella infections are initiated through the per os route with the ingestion of contaminated food or water. The bacteria travel to the gut and infect the M-cells in the Peyer's patches via fimbria. SPI-1 and SPI-2 pathogenicity islands are responsible for the genes produced which are necessary for the invasion of the intestinal epithelial cells together with the formation of the systemic infection and the production of intestinal inflammatory secretions and responses. Cell internalisation of *Salmonella* happens rapidly (within minutes) of contact. This is followed by inflammation of the mucosa in addition to the accumulation of neutrophils and macrophages which lead to the manifestation of a secretory diarrhoea. Bacterial effector molecules are the main culprit of intestinal membrane ruffling likely due to inositol-signaling pathways causing remodelling of actin cytoskeletons. Other virulence factors are also responsible for the overall outcome of the infection and pathogenesis of *Salmonella*, they determine whether the disease can result into septicaemia or not (Ettinger et al., 2017).

In most cases canines infected by *Salmonella* are concurrently infected with 2 or more serotypes. Up to four serotypes were identified within the same animal at the same time therefore complicating the infection. Pathological investigations carried out on dogs with confirmed salmonellosis have found characteristic lesions including: catarrhal,

hemorrhagic enteritis in addition to mucosal sloughing and intermittent denuded portions of the gut. Presumably shedding of naturally existing *Salmonella* infections happen for a minimum time period of 6 weeks and is harboured in the lymph nodes, making it possible for shedding to happen for such an extended time. Carriers of *Salmonella* may not shed the bacteria, however in the case of co-infection (ex.viral-enteritis) or when a canine is under stress, the bacteria may move out of the lymph node and be shed again together with faecal excretes (Morse et al., 1976) .

2.5 Canine salmonellosis as a risk of zoonosis

In numerous parts of the world, family pets have posed a risk to transmitting zoonoses among members of the family. The exact degree of infection caused by household pet zoonoses is not exactly known, however more than 70 pathogens of companion animals which can possibly infect humans have been recorded. Living in close proximity with companion animals has in fact been confirmed as a potential risk factor of infection with pathogens, for some species the risk is higher than others (Hashemi et al., 2016).

The occurrence of the infection as well as the carrier state of *Salmonella* bacteria within dogs is of great importance and risk to public health, especially because of the close contact between canines and their owners (Sato et al., 2000). “Salmonellosis was the second most reported zoonotic disease in humans in 2008” (Hashemi et al., 2016).

Human salmonellosis is contracted by the faecal-oral route mainly by the handling and consumption of contaminated food of animal origin (the majority being meat, eggs, poultry and milk) and water, while rarely being transmitted from person to person (World Health Organization, n.d.). Several studies done around the world have revealed that canines may serve as a potential source of human salmonellosis, in fact potential episodes of canine to human transmission of *Salmonella* have involved several serotypes (Morse et al., 1976). Pets as a cause of human salmonellosis, including: chickens, dogs, cats, rabbit, rodents, ferrets, turtles and other reptiles are held responsible for 15-20% of the total cases of infection. It is also approximated that 1% of infections are caused by companion animal contact within the USA. Infection from pets can be due to direct or indirect contact with them, for example contact with water in which the pet has swum or drank from (Wray and Wray, 2000). “Activities involving close contact between dogs and people include

sleeping, playing, eating, greeting, the disposal of faeces, and general physical contact through tokens of affection such as cuddling and stroking” (Westgarth et al., 2008).

Even though *Salmonella* exists within the environment, numerous serotypes of *Salmonella* (besides *S. Typhi*) usually inhabit animals that do not show any clinical signs or symptoms. Although any age group is susceptible to being infected with *Salmonella* by contact with infected animals, in view of recent studies it is noted that the likelihood of zoonotic infection from canine salmonellosis is more likely to be contracted by infants, children, the elderly and immunocompromised individuals (Hashemi et al., 2016). In justification of this Sato et al. (2000) published a case report of an infant which had diarrhoea and which was living in the same household and in close proximity of two dogs. *S. Virchow* was isolated from the infant’s faeces and the findings revealed that it was likely that the infected infant manifested the infection from the dogs themselves due to the fact that the same pathogen was found in the canine faecal samples. Antibiotic sensitivity and PFGE patterns were very much alike in the respective isolates. Sato et al. (2000) explained that the infectivity of the *Salmonella* within the canines’ faeces was high and that they were likely infected from eating contaminated chicken meat or from environmental infection while on a walk. The 4 month-old infant was said to be infected the moment one or both of the dogs was within a carrier state of salmonellosis and consequently shed and transmitted the disease through environmental contamination (Sato et al., 2000).

The discovery of *S. enterica* serotype Havana in a 2.5 months old infant which developed gastrointestinal illness prompted the examination of commercially prepared dehydrated dog foods as being the potential source of infection. It was learned that the canine companion animal had fell ill and was treated for “digestive disruption”. Even though bacteriological isolation was not done at the time, a specimen collected later on (January 13, 1976) confirmed the dog to be positive for *S. enterica* serotype Newington. Although the study suggests that mishandling and contamination with *Salmonella* infected commercial dry food is the cause of the disease in humans, this study conducted by Pace et al. (1977) further strengthens other publications suggesting that salmonellosis in dogs increases the risk of transmittance of infection to humans (Pace et al., 1977).

Animal assisted therapy is becoming an increasingly popular mode of therapy for patients within hospitals and care homes. Speculations about the health of patients being

jeopardised due to risk of zoonotic infection, especially those which are in an immunocompromised state within these therapeutic sessions, have arisen. A study by Lefebvre et al. (2008) investigated this concerning matter by sampling “faeces, hair coat brushings and one rectal, aural, nasal, oral and pharyngeal swab” from 102 pet-assisted therapy (PAT) dogs for 18 specific zoonotic agents. The dogs were subjected to a routine physical examination while the owners were interviewed in the form of a questionnaire. All the canines were deduced to be clinically healthy during this intervention; however zoonotic agents were found harbouring 80 out of 102 dogs (80%). In total, 3% of the dogs were confirmed to be positive for infectious agents within the genus *Salmonella* (Wray and Wray, 2000).

In a separate study two hundred healthy PAT dogs were part of a Canadian investigation. Every two months for a total of 1 year, faecal samples from the PAT dogs were acquired for the purpose of culturing zoonotic agents. Together with the faecal samples a record was kept of the “places visited, antimicrobial use within the home, dog health status and diet.” It was concluded that the dogs (20% of 200) which were fed raw meat within some instances during the year, had a higher incidence rate of salmonellosis than the rest of the population. Therefore it was recommended to prevent dogs which are kept on raw food based diets to be enrolled in PAT programs, particularly when they are interacting with immunocompromised individuals which have an increased risk of contracting zoonotic agents (Wray and Wray, 2000).

It is of utmost importance to educate owners on keeping hygiene levels at an optimum level when keeping dogs (and other species in general) within a household. This idea is strengthened in a case report of a dog to child transmission of *S. Enteritidis*. A dog which consumed chicken broth (which remained on the kitchen stove for “one or two days”) manifested “severe nausea, vomiting, and a fetid, profuse diarrhoea”. The child was residing in close proximity to this dog and its contaminated environment at the time, subsequently suffering an acute illness. *S. Enteritidis* isolated from the dog had an indistinguishable antimicrobial sensitivity pattern to that of the child with one special case: the dogs isolate resisted sulfonamides unlike the child’s. It is highly probable that the child was infected through being in close proximity to the infected household dog, this opinion is further reinforced by the fact that the child was teething at the time therefore increasing

susceptibility for infection (Morse et al., 1976). In separate cases, *S. Enteritidis* phage types 1 and 8, *S. Typhimurium* DT104 and *S. Indiana* were also isolated from dogs which were not showing any clinical symptoms and which were regarded as clinically healthy, however thought to be the source of infection for human beings manifesting diarrhoea due to the analogous strains (Philbey et al., 2013).

Spread of diseases to man is not a straight-forward matter. It usually requires repeated close contact with household pets and their products of excretion. It is important to keep a good hygienic practice so as to minimise this risks, especially when the dogs are in contact with children, old people and immunocompromised patients. It is also fundamental to avoid feeding raw meat to these animals (Hashemi et al., 2016). Human pregnancy has also been a factor indicated to increase susceptibility to being infected with *Salmonella*, hence extra precautions should be taken (Morse et al., 1976). To conclude, the isolation of *Salmonella* in clinically healthy or sick dogs could be of major epidemiological importance and can have severe impacts on disease control programs as these animals may serve as a potential source of contamination to the human environment including their food and water (Akwuobu et al., 2018).

2.6 Treatment and prevention of *Salmonella* infection

In order to reduce risks of *Salmonella* infection to a minimum for both dogs and humans, it is of great importance to maintain a standard of environmental and personal hygiene. It is reliant on the avoidance of primary or secondary contact or contamination with infected matter or infected beings (Morse et al., 1976). This is especially emphasised within households which keep dogs in close contact with humans and also other animals as the probability of contracting an infection is higher in these cases. Dogs must be withheld from coprophagic behaviours and should be fed well-prepared and properly cooked food to prevent contamination with *Salmonella* (Akwuobu et al., 2018).

Another important point set up in a study by Pace et al. (1977) is the importance of creating awareness to consumers in connection with dog food being a possible vector of *Salmonella* contamination within their home environment and infection to humans/dogs. It is critical to warn consumers that dog food may be contaminated with *Salmonella* and that it should be handled with care and appropriate hygiene. In this study products of two

manufacturers were identified to contain *Salmonella*, therefore Pace et al. discussed that manufacturers of dog food should make laboratory testing a priority to reduce consumer risk to a minimum by establishing critical control points in the production of dried food through monitoring parameters like time, temperature and moisture contents (also keeping in mind post-processing opportunities of contamination). Although one does not intend to consume dog food, it is apparent that handling of dog food and being in proximity to dogs infected with *Salmonella* are also a threat for disease transmission to humans (Pace et al., 1977). Contaminated home-cooked foods fed to companion animals may also serve as a perfect medium of infection to humans, multiplication in food matter kept at room temperature for several hours is very probable therefore it is advised to keep hot foods above 60 degrees Celsius and cold foods at 4 degrees Celsius to reduce the risk factors (Morse et al., 1976).

Present protocols for the management of clinical salmonellosis in dogs do not suggest the use of antimicrobial agents for uncomplicated cases. In opposition to this, it is essential to use antimicrobial agents in cases of systemic infection or when an immunocompromised animal is infected, to prevent further complications (Mark et al., 2011). The study conducted by Philbey et al. (2013) emphasises the difficulty on decision making when prescribing and choosing the right antimicrobial agents for cases of clinical salmonellosis in dogs. The responsibility of choosing the right antimicrobial agent preventing further multi-drug resistant bacteria is essential. The dilemma highlights the obligation for culture and antimicrobial sensitivity testing in the management of clinical salmonellosis in veterinary practice (Philbey et al., 2013). Supportive and symptomatic therapy is of essence, fluids must be administered to prevent dehydration, avitaminosis and electrolyte imbalance and to avoid an overall malnutrition status within the animal. Efforts to reduce symptoms of diarrhoea, pyrexia, nausea and vomiting are also designated. “There is no substitute in the salmonellae-infected patient for good nursing care as well as clinically indicated supportive and symptomatic therapy” (Morse et al., 1976).

3. Aim of investigation

The aim of my investigation was to identify the frequency of Maltese hunting and scavenging dogs (owned by local hunters and/or farmers) harbouring *Salmonella* within them. The investigation took place at various randomly picked locations within Malta so as to get a representative sample of the whole local hunting and scavenging canine population.

In the summer of 2018, I had opportunity to collect single rectal samples from dogs kept on pig farms, chicken farms and dairy farms. Part of their purpose on these farms was to hunt pests like rats, however in many cases these dogs usually ended up catching a number of other different species (as by-catch) entering the premises while also being used to accompany their owners during the hunting seasons. A great amount of the dogs tested for the purpose of this study were canines primarily kept just to assist local hunters to catch wild birds or wild rabbit. The local hunting scene is very popular among the Maltese population, in fact twice a year in spring and autumn the hunting season opens and Maltese hunting dogs accompany their owners to retrieve targeted carcasses of game from wheat fields. These dogs kept for hunting and scavenging purposes, frequently join their owners at home after a day of hunting.

The study could eventually shed a light on the risks of keeping these dogs within households and in close contact with humans and/or other animals as a mode of spreading salmonellosis. Keeping dogs positive for *Salmonella* in proximity to humans could in fact be of a public health concern and this study may be essential for control and management of the disease affecting animals, humans and public health in Malta.

4. Materials and Methods of experiment

4.1 Study area

The study areas investigated within this project were various locations within Malta which are well known to accommodate a high density of farmers and hunters usually within the more rural areas of Malta. In order to obtain maximum epidemiological coverage, many of the locations chosen in this study were scattered around many different geographical areas on the island. The dogs had contrasting housing environments including living in private households (n=25), pig farms (n=12), chicken farms (n=5) and dairy farms (n=8). Owners volunteered for this research upon personal contact after explaining the whole process and the purpose of this study. Below is a map of the specific villages and places where the samples were taken. Informed owner consent was gathered for all dogs which were part of this study (Figure 4).



Figure 4

Map showing the locations of sample collection

Source: Google Maps

4.2 Sample size and sampling

Sampling of the chosen dogs was split over several days, in compliance with the availability of the dog owners within the summer of 2018. In total, 50 dogs participated within this study ensuring a representative sample of the whole Maltese hunting and scavenging dog population. The age of the animals being tested for bacteriological sampling ranged between 9 months to 12 years old and of the dogs tested, 32 were female and 18 were male. Samples were tested for *Salmonella* using standard and enrichment culture-based method. The total time for the average sampling and testing procedure took 48 hours.

4.3 The swabbing procedure

Using gloves and plastic handled sterile swabs, faecal specimens were collected by rectally swabbing each canine included within this study. It was impossible to collect all 50 samples within one day due to owner compliance, therefore the sampling was split into several sessions on different days. Sample time was kept to a minimum so as to avoid stressing the animal out. The swabs were immediately inoculated into vials containing Rappaport-Vassiliadis enrichment broth (product by Oxoid Microbiological Products, Code: CM0669), properly sealed and placed within a case keeping the samples at ambient temperature. Each sample was labelled with a specific code, so that each canine sampled could be properly traced.

4.4 *Salmonella* selective-enrichment

Rappaport-Vassiliadis (RV) is a selective enrichment medium which can be used for isolating *Salmonella* species from various samples including food and different environmental specimens. In accordance with the original formulation characterised by Rappaport et al. (1956) the broth used takes advantage of the four unique characteristics of *Salmonella* species in comparison to other Enterobacteriaceae so as to have a successful selection and isolation:

1. *Salmonella* can survive and be resistant to high osmotic pressures.

2. *Salmonella* is able to multiply at relatively low pH values.
3. *Salmonella* can resist Malachite green to a greater extent.
4. *Salmonella* is less nutritionally demanding than other Enterbacteriaceae. (Oxoid.com, n.d.)

Within a few hours of collection, the samples were taken to the Laboratory (BioDNA Laboratory Services Ltd.) so as to be incubated for 24 hours at an incubating temperature of $42 \pm 1^\circ\text{C}$.

4.5 Inoculation on agar plates

After 24 hours within the incubator, the selective enrichment medium of each sample was inoculated onto a *Salmonella* chromogenic agar plate (product by Condalab CAT: 1122.00). This agar is a selective chromogenic medium traditionally used for the detection of *Salmonella* species from clinical samples, foods and water. This type of medium is optimal in order to differentiate physiological bacteria within the Enterobacteriaceae family from *Salmonella* species which may be pathogenic. These media were routinely used to identify *Salmonella* from other Enterbocteriacea based on two special characteristics of the species:

- The ability of *Salmonella* to produce hydrogen sulfide.
- The inability of *Salmonella* to ferment Lactose.

However, the above two characteristics have not always been successfully in the proper identification of *Salmonella*, as there are over 2000 species of *Salmonella* which do not share the same two properties.

The principle ingredients present within this agar and their main purposes are listed within Table 2.

This particular chromogenic agent is based on the combination of two different chromogenic substrates that aid in the identification of *Salmonella*:

- A. In case the presence of *Salmonella* within the sample is positive, the bacteria will be evident as magenta coloured colonies. The *Salmonella* positive reaction is a result of hydrolysis of one of the chromogenic substrates contained within the medium, as a result of their inability to utilise the alternative chromogenic substrate.

B. If there are other microorganisms present within the gut, they will cleave the other chromogenic agar and as a result of this they produce blue-green coloured colonies.

Main ingredients	Main objectives of the ingredients
Casein Peptone and beef extract	Providing nitrogen, vitamins, minerals and amino acids essential for growth
Chromogenic mixture and Sodium citrate	Aids in the inhibition of Gram-positive organisms, Proteus and Coliforms
Bacteriological agar	The solidifying agent
Inhibitory Supplement	Inhibits accompanying flora which may result in false positive results

Table 2

The next step of the process was incubating the inoculated medium at a temperature of 35 ±2 °C and left to culture for 18-24 hours. The colour of the colonies were then examined for the possibility of magenta *Salmonella* colony growth. If magenta coloured colonies were present, further confirmation with PCR was necessary (Condalab, n.d.).

4.6 *Salmonella* spp. confirmation and serovar typing: Enteritidis and Typhimurium

The following process was used to confirm the presence *Salmonella* and for typing its serovar:

1. DNA was extracted by adding the magenta colony to 150ul Tissue Lysis buffer and incubating the tube at 85°C for 20 minutes.
2. For the PCR, a master mix was prepared as described in Table 3., using primer sequences described in Table 4.
3. Following preparation of the master mix, a 22.5ul aliquot was transferred to 0.25 mL PCR tube.
4. To the master mix (22.5ul in each tube), Sample (Table 5) or Non template control or Positive Control DNA were added.

Salmonella Multiplex Mastermix			
Reagents		1x 25µL Reaction	For ___ Samples
5x buffer B1 + MgCl ₂		5 µL	
dNTP mix	2.5nM	2 µL	
<i>Taq</i> polymerase	5U/ µL	0.5 µL	
Primer	*Salm. primer mix	1.5 µL	
Water	--	13.5 µL	
		Total: 22.5µL/reaction	

Salm. Primer Mix	Use 1:1:1 mix of INVA ,IE1, FLICC 50pmol/ µL primers
-------------------------	--

Table 3

Primer Sequences:	
The primers were purchased from Bioneer and had the following sequences:	
INVAF	CGGTGGTTTTAAGCGTACTC
INVAR	CGAATATGCTCCACAAGGTT
FLICCF	CCCGCTTACAGGTGGACTAC
FLICCR	AGCGGGTTTTCGGTGGTTGT
IE1F	AGTGCCATACTTTTAATGAC
IE1R	ACTATGTCGATACGGTG

Table 4

Sample	NTC	2.5µl (DNA Buffer)
	Sample DNA	2.5µl (Extracted DNA)
	Positive Control DNA	2.5µl (Positive Control)

Table 5

5. The tubes were labelled in a thermal cycler. Table 6 describes the PCR thermal cycling program, used for the PCR.

PCR thermal-cycling:			
Stage	Temperature/ °C	Time	Cycles
Taq-Activation	95	15:00	1
Denaturation	95	1:00	30
Annealing	58	1:00	
Extension	72	0:30	
Final extension	72	7:00	1

Table 6

6. After PCR the samples are loaded on a pre-stained (ethidium bromide stain), 1.5% agarose gel.
7. Following the running of the gel, the bands observed are interpreted as shown in Table 7.

Salmonella PCR result			Inferences
INVA (796bp)	FLICC (432bp)	IE1 (316bp)	
-	-	-	Negative for <i>Salmonella</i> spp.
+	-	-	Positive for <i>Salmonella</i> spp. not Typhimurium or Enteritidis
+	+	-	Positive for <i>Salmonella</i> Typhimurium
+	-	+	Positive for <i>Salmonella</i> Enteritidis
+/-	+/-	+/-	DNA extraction unsuccessful – Repeat Procedure

Table 7

5. Results

A total of 6 out of 50 *Salmonella* chromogenic agar samples were suspected to be positive for the presence of *Salmonella* due to the presence of magenta colonies. Below are a few photos of the *Salmonella* chromogenic agars after being cultured:

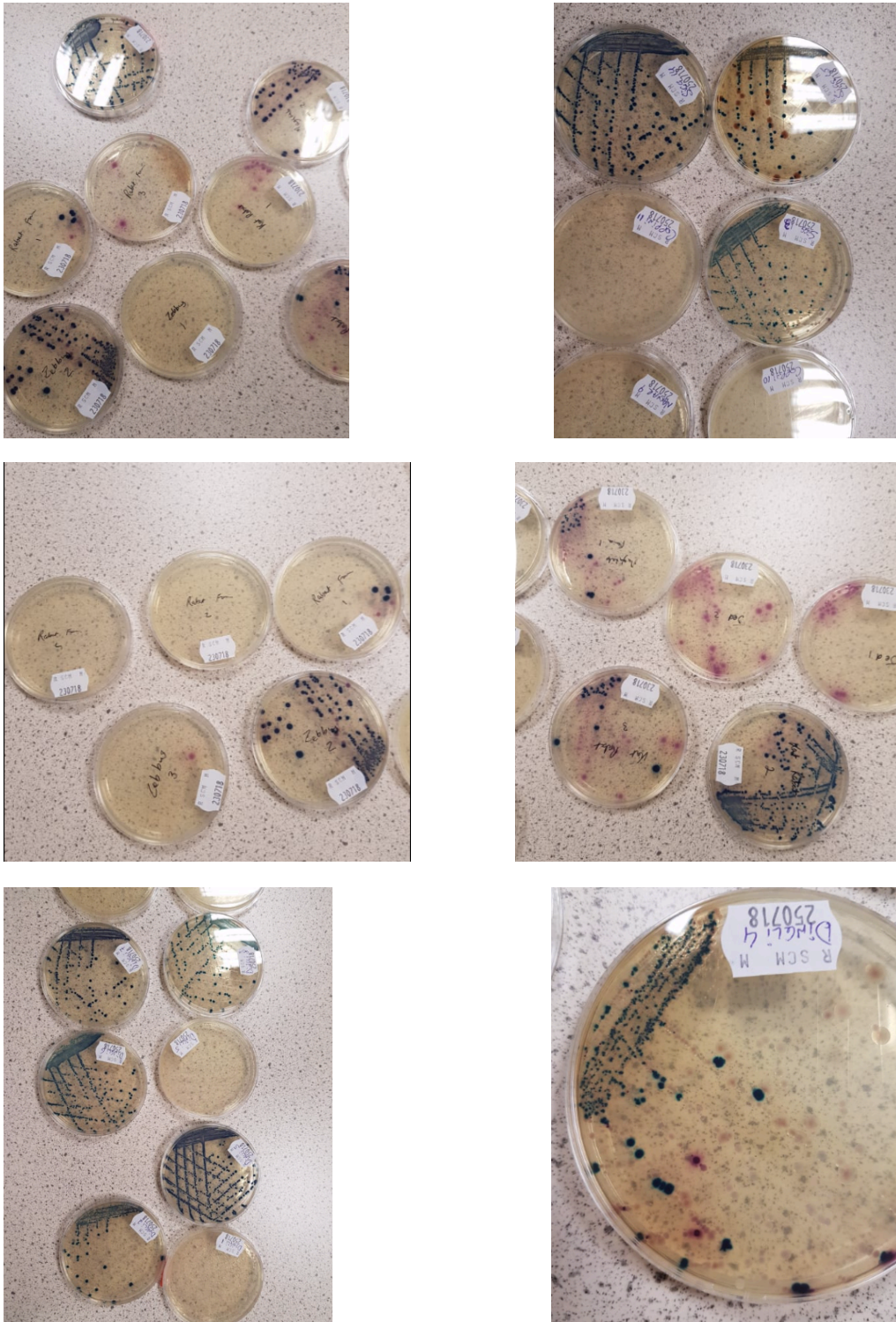


Figure 5
Suspected magenta colony

The suspected samples were then tested for *Salmonella* spp. and serotyping using PCR, the results of this investigation being presented in Table 8, below:

Locality sampled	Main area of hunting activity	Sex	Age (years)	<i>Salmonella</i> status
Mgarr	Game	Female	1.5	Negative
Mgarr	Game	Male	7	Negative
Mgarr	Game	Female	6	Negative
Mgarr	Game	Male	2	Positive
Mgarr	Game	Female	4	Negative
Mgarr	Game	Female	4	Negative
Mgarr	Game	Female	8	Negative
Mgarr	Game	Female	10	Negative
St.Pauls Bay	Dairy farm	Male	1	Negative
St.Pauls Bay	Dairy farm	Male	0.75	Negative
St.Pauls Bay	Dairy farm	Female	3	Negative
Rabat	Pig farm	Male	8	Negative
Rabat	Pig farm	Female	8	Negative
Rabat	Pig farm	Female	8	Negative
Rabat	Pig farm	Female	3	Negative
Rabat	Pig farm	Female	2.5	Negative
Rabat	Pig farm	Male	1	Negative
Rabat	Game	Male	5	Negative
Rabat	Game	Female	6	Negative
Salini	Chicken farm	Female	11	Positive
Salini	Chicken farm	Female	9	Negative
Salini	Chicken farm	Female	9	Negative
Naxxar	Game	Male	2	Negative
Naxxar	Game	Female	3	Negative
Naxxar	Game	Female	3	Negative
Hal- Gharghur	Game	Female	11	Negative
Hal- Gharghur	Game	Female	9	Negative

Locality sampled	Main area of hunting activity	Sex	Age (years)	Salmonella status
Hal- Gharghur	Game	Male	12	Negative
Haz-Zebbug	Pig Farm	Male	7	Negative
Haz-Zebbug	Pig farm	Male	3.5	Negative
Haz-Zebbug	Dairy farm	Female	3.5	Negative
Haz- Zebbug	Dairy farm	Female	6	Negative
Had- Dingli	Dairy farm	Female	6.5	Negative
Had- Dingli	Game	Female	1	Negative
Had- Dingli	Game	Male	1	Negative
Had- Dingli	Game	Male	3	Negative
Had- Dingli	Pig farm	Female	4	Negative
Had- Dingli	Pig farm	Female	3	Negative
Had- Dingli	Pig farm	Female	12	Negative
Had- Dingli	Pig farm	Male	9	Negative
Siggiewi	Game	Male	3	Negative
Siggiewi	Game	Male	2	Negative
Siggiewi	Game	Female	2	Negative
Siggiewi	Game	Female	1	Negative
Marsaskala	Dairy farm	Male	4	Negative
Marsaskala	Dairy farm	Male	6	Negative
Zurrieq	Game	Female	3	Negative
Zurrieq	Game	Female	2	Negative
Zurrieq	Chicken farm	Female	6	Negative
Zurrieq	Chicken farm	Female	5	Negative

Table 8-Data on canines tested for Salmonella

As shown in the table above, two out of 50 rectal swabs tested were positive for *Salmonella* species. However the possibility of them being *S. Typhimurium* or *S. Enteritidis* has been excluded.

6. Discussion

The apparent incidence of faecal *Salmonella* shedding among Maltese hunting and scavenging dogs was 4% of the population tested. This study was a first of its kind in Malta and it has greatly improved our knowledge on the *Salmonella* incidence within these canines, revealing that in fact dogs within the Maltese islands are able to harbour and shed *Salmonella* putting the public at risk. The result of this study was as expected considering that these canines are being continually exposed to a variety of contaminated environments and possibilities of infection. On the contrary there were many limitations present with the method of analysis used within this investigation therefore it was very possible to get a differing result from the true value.

The methods used to isolate *Salmonella* within this study shed a light on the minimum number rather than a maximum number of isolations possible. Faecal samples are not easy to obtain from dogs having this lifestyle as most of them are not in contact with many other humans, besides their respective owners. Rectally swabbing these canines was a challenge in itself and it was only possible with the handling and help of their owners and more often than not, it was a rectal swab with only a minute amount of faecal material attached.

The limitations of single faecal cultures for *Salmonella* detection are well documented in other studies (McKenzie et al., 2010), in fact faecal culturing does not have perfect sensitivity for the successful detection of *Salmonella* within a sample, therefore this result may be an underestimate of the true value of its actual prevalence. Another important aspect that shall be mentioned is the fact that each dog was only tested on one occasion. Taking into consideration that *Salmonella* is usually being intermittently shed for several weeks, many of the dogs tested may have been harbouring *Salmonella*, yet not concurrently shedding the pathogen. This may be due to many factors including the animals immune status and the present stage of *Salmonella* infection. Therefore, duplication of tests, media and selective plating would have increased the probability of positive samples, as has been discovered in many other studies on *Salmonella*.

Sampling of a greater number of dogs would have resulted in a more precise survey and a more representative study about the factual status regarding the prevalence of *Salmonella* within these dogs. However, it turned out to be a greater challenge than previously perceived in order to collect a higher amount of samples from the hunting and scavenging

dog population. This was mainly due to owner compliance and scepticism about the study, despite the fact that the owners were given a thorough explanation about the process of sampling and it being minimally invasive together with the aims of the study.

Salmonella incidence rates across the globe have varied greatly when conducting similar studies. Factors affecting the resulting outcomes of each study include: the conditions of the animal population, the geographical location of the animals, the time of sampling, the sample size taken, the sampling strategies and the isolation methods performed (Seepersadsingh et al., 2004). Among different canine populations, the incidence of *Salmonella* shedding ranges from approximately 1-20% for pet dogs (Hackett and Lappin, 2003; Leonard et al., 2011; Procter et al., 2014) while on the other end of the scale, sled dogs and racing greyhounds have been found to have a higher prevalence ranging from 70-90% (Morley et al., 2006; McKenzie et al., 2010).

Although the prevalence rates of *Salmonella* status in canines varies between different countries, it is of interest that the observed prevalence of 4% within the Maltese islands may be compared with other studies conducted worldwide. The percentage of positive cases exceeded that of other studies, for example that of Trinidad (3.6%) (Seepersadsingh et al., 2004), Italy (2.4%) (Natasi et al., 1986) and Northern Bavaria, Germany (3.45%) (Bagcigil et al., (2007). The result only differed by 0.3% from that discovered from household and stray dogs tested in Taiwan (4.3%) (Tsai et al., 2007). On the contrary, the incidence rate differed significantly from that of the asymptomatic greyhounds bearing *Salmonella* (11%) tested by Stone et al. (1993). Other canines which also had a significantly higher prevalence rate were the dogs tested in Tehran, Iran (22%) (Hashemi et al., 2016) and the racing sled dogs tested by Cantor et al. (1997) where 63% of the dogs tested positive for *Salmonella*.

The prevalence of *Salmonella* has been reported to be more common in stray dogs rather than in household dogs according to the literature (Tsai et al., 2007). This may be due to a fundamental factor which may be altering the *Salmonella* status of a canine- the diet. In a study performed on racing greyhounds, 44% have been found to be shedding *Salmonella* sub-clinically (Stucker et al., 1952) while in a more recent study 11% shed *Salmonella* asymptotically (Stone et al., 1993). It is thought that their *Salmonella* status may have originated from their diet being a high-protein raw meat diet. This was further confirmed

when identical enterotypes were found in the food and within the faeces of those canines which consumed the raw meat (Lowden et al., 2015). In contrast to this, none of the retired-greyhounds tested by Lowden et al. (2015) within the Midlands region of the United Kingdom tested positive for *Salmonella*, this may be a result of them being fed commercial diets.

The genus *Salmonella* consists of more than 1300 different serotypes, all being potential pathogens. Some of these serotypes are uniquely species-specific, for example *S. Typhi* and *S. Paratyphi* affect only man. However other *Salmonella* species such as *S. Typhimurium* are not species-specific and generate disease in man and a variety of different animal species (Fischer et al., 1984). Within this study, canines residing on farms were in contact with different animals and livestock, this factor may have been one of the main culprits of contracting salmonellosis. One of the canines which resulted to be positive for harbouring *Salmonella* was residing on a fancy chicken farm and therefore it was in contact with them and their faecal matter which may have been contaminated with *Salmonella* and transmitted to the dog. The second dog which was positive for shedding *Salmonella* was a hunting dog which was used for the retrieval of dead animals back to the owners, possibly contaminated. It is likely that 4% of the dogs have been found positive for *Salmonella* due to the fact that they are left to scavenge for themselves throughout the day, exposing them to various environmental contaminants including faeces and carcasses of other animals which may be harbouring *Salmonella* within them.

It is also important to note that the incidence rate of *Salmonella* prevalence may have decreased in comparison to the past hunting and farm canine populations due to the recent increase in the feeding of modern commercialised processed foods rather than allowing the animals to eat left over scraps and scavenge all day long. However this can not be proven as yet, as no previous studies done in Malta regarding this matter were found. In spite of commercialised food being manufactured in such a way as to minimise the risk of *Salmonella* contamination, *Salmonella* incidence was also reported in dogs and humans due to contamination of commercial food sources (Lowden et al., 2015).

Considering that *Salmonella* may be transmitted from dogs onto their human counterparts, as confirmed in many studies conducted worldwide including a report completed by Morse et al. (1976) about a dog to child transmission of *S. Enteritidis* (Akwuobu et al., 2018).

This study reveals that these canines may be a risk of severe infection to the Maltese public. Dog owners who keep dogs for hunting/scavenging purposes on farms or within households and which have frequent contact with their pets should be aware of this fact so as to take the necessary precautionary measures. The results obtained in this study were two positive canines for *Salmonella* spp. but neither of the serotypes were *S. Enteritidis* or *S. Typhimurium*. However, these two serotypes are the most frequently isolated serotypes of *Salmonella* in both humans and animals in the UK (Lowden et al., 2015). Concurrently a study conducted in Australia shows that canines most frequently harbour *S. Typhimurium* (Simpson et al., 2018), so the discovery of other *Salmonella* species in this study is atypical. The exact serotype could not be identified due to the laboratory limitations.

The dogs included within this study were apparently healthy and did not show any abnormal clinical signs which could indicate a pathogenic infection, according to their respective owners (even though two of the dogs tested resulted in being positive for harbouring *Salmonella*). *Salmonella* may be transiently passing through the intestinal tract in many cases but no colonisation would be present, therefore making them passive-carriers of food-borne *Salmonella* (Lowden et al., 2015). The most frequent form of *Salmonella* in dogs is the subclinical carrier state in which the canine is able to shed the bacteria and contaminate the environment, but does not show any significant clinical signs (Sanchez et al., 2002). Nonetheless, young dogs may be more susceptible to developing clinical signs due to their underdeveloped immune system and additionally more likely to result in septicaemia (Morse and Duncan, 1975; Mcgavin et al., 2001). In clinical instances of salmonellosis in dogs, morbidity could be close to 100% in puppies while mortality can approach 40% (Mcvey et al., 2002). Within this study there was no significant difference on infection status depending on age, as both dogs which resulted to be positive for *Salmonella* were of a relatively median age.

In conclusion, *Salmonella* isolation from hunting and scavenging farm dogs is of public health importance. These canines may be a source of disease transmission to the community as many serotypes are considered highly virulent to the human host. Apart from this, due to them being in close contact with livestock in some instances, they may also pose a risk to the safety of the food-chain by introducing a source of Salmonellosis on a food-producing farm.

7. Conclusions and Recommendations

The occurrence of *Salmonella* within these canines considering the variety of risks they are susceptible to, is on the lower side of isolation rates in comparison with some other countries where analogous studies were made. However the result is still significant as *Salmonella* shedding was still identified considering the low number of animals tested and limitations present. These dogs may still pose a risk to the public and other animals they may get in contact with, therefore it is recommended that routine controls shall recommence in the near future. Public education and awareness regarding suitable hygiene practices, such as frequent hand disinfection can help reduce the possibility of *Salmonella* infections acquired from dogs and equally from surrounding sources (Lowden et al., 2015). Proper management to prevent faecal contamination, including contamination of food and water is essential to prevent the spread of disease. Dogs shall be prevented from eating any faecal matter and shall be provided with uncontaminated and properly cooked food (Akwuobu et al., 2018). By reason of the results obtained in this study, it is also suggested to prevent frequent direct contact between dogs and livestock along with their excretions.

The idea that dogs may act as reservoirs or vectors for *Salmonella* is of concern for human health for 2 main reasons. Firstly, antibiotic resistance patterns may be altered due to frequent treatment with them. Secondly, the intimate relationship between companion animals and humans frequently includes direct-contact (licking) and sharing of the immediate environment, heightening the risk of transmission (Simpson et al., 2018).

In conclusion, it is thought that hunting and farm dogs may be an important carrier of *Salmonella* and a source of human salmonellosis in Malta. Additional studies must be performed so as to establish the epidemiological relationship between salmonellosis in canines, their owners and other surrounding animals. It would be beneficial to repeat the study in the near future with a larger sampling population and repeated samples taken from the same animal in order to achieve a more representative and clear result, discovering the exact serotype would also be of great value for a similar study. Furthermore, these studies may conclude whether the *Salmonella* status may have an effect on surrounding livestock and human-beings living in proximity to them.

8.Summary

Objective: The purpose of this study was to initiate an investigation to ascertain the incidence of *Salmonella* shedding by apparently healthy Maltese hunting and scavenging dogs, while highlighting the risks which may affect dog-owners and their livestock.

Materials and methods: Rectal swabs from 50 dogs dispersed in different locations around Malta were examined, each test being done only on one occasion. The samples were cultured for *Salmonella* using Rappaport-Vassiliadis selective enrichment broth (Oxoid CM 0669) and *Salmonella* chromogenic agar (Condalab CAT: 1122.00). Suspected *Salmonella* isolate samples then underwent *Salmonella* species confirmation and serovar typing, specifically for *S. Enteritidis* and *S. Typhimurium*.

Results: Two of the Fifty samples taken were positive for *Salmonella*, this means a total prevalence of 4%. PCR confirmed that the two *Salmonella* isolates were neither *S. Enteritidis* or *S. Typhimurium* although the serotype was not confirmed.

Conclusion: The isolation of salmonellae in apparently healthy hunting and scavenging dogs was low, however the result is still significant and indicates a carrier status which may pose a risk to the disease control in the surveyed area. The contaminated game-meat or livestock may be the main source of infection. These results could be useful in planning control and preventative programs associated with this dog population.

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