Seroprevalence and Microbiological Monitoring in Eggs for Salmonella enterica Serovar Enteritidis and Salmonella enterica Serovar Typhimurium in Ornamental Chicken Flocks in Italy

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INTRODUCTION

Salmonellosis is a significant zoonotic disease with a considerable economic impact on the production of eggs and in general, on the whole poultry industry. According to the European Food Safety Agency (EFSA), Salmonella spp. is one of the top pathogen agents involved and more distributed in European Union (EU) in foodborne outbreaks (EFSA, 2008; WHO, 2017), and is the third cause of death among food transmitted disease (Ferrari et al., 2019). Some serovars, such as Salmonella Pullorum and Salmonella Gallinarum are typical of birds, causing disease in animals but rarely in humans, while others such as Salmonella enterica serovar Enteritidis (S.E.) and Salmonella enterica serovar Typhimurium (S.T.) are able to infect a broad range of hosts (Ferrari et al., 2019) S.E. and S.T. are the serovars more associated with gastrointestinal illness in humans, but also other serovars can cause disease and represent a public health issues, such as S. Infantis (Vieira et al., 2008; EFSA, 2010; Abd El-fatah et al., 2020). Across the EU, 32% of Salmonella cases reported are caused by S.E., 16% from S.T. and 6% caused by other Salmonella species, especially from S. Infantis. In Italy, according to the reports of EFSA (2018, 2019), the percentage of positivity for zoonotic Salmonella spp. such as S.T. and S.T. monophasic variant
(S.T. sv) in industrial chicken flocks is 0.2%, while in laying hens is 0.3%. Overall, the prevalence of Salmonella spp. in poultry industry remains lower than 1%. Surveillance programs and intervention strategies to control foodborne salmonellosis have been implemented in EU Member States, although a clear evaluation of the effect of such interventions is difficult. From a public health perspective, there is inherent risk correlation between zoonotic transmission of pathogens and poultry husbandry and production. In the last years in Italy, the breeding of ornamental poultry for self-consumption of eggs and meat, for beauty competitions and to preserve local breeds, has regained popularity. Often, backyard poultry farmers have limited knowledge of bio-security practices and are not included in vaccination schedules or monitoring plans, as indicated on the Regulation 2160/2003 (European Commission, 2003) and 13.11.2013 (Ministry of Health, 2013). In particular, farms with less than 50 birds or with a number of birds between 50 and 250 (reared without commercial purposes but only for self-consumption) are not included in the National Control Plan for Salmonella (NCPS).

In general, the Italian legislation on Food Safety exclude all products intended for self-consumption from official controls, as indicated in the Regulation 178/2002 (European Commission, 2002). However, according to Regulation 1308/2013 (European Commission, 2013), eggs produced by rural farms can be sold in local markets or within 10 kilometres from the sites of production without weight classification, otherwise indicated in the Regulation 1234/2007 (European Commission, 2007) and 589/2008 (European Commission, 2008). To our knowledge, there are limited information about the prevalence of Salmonella spp. in ornamental poultry flocks in Italy, also by the scarcity of data that would be obtained from an adequate monitoring plan. In other countries, such as in South Australia in a study conducted by Manning et al. (2015), 30 backyard poultry flocks were screened for Salmonella spp. and 4 tested flocks resulted positive. The overall Salmonella spp. isolation rate in the study was 10.4%, with a prevalence at individual bird level of 0.02%. In Finland, S. enterica was only found sporadically in feral and environmental samples of backyard poultry (Pohjola et al., 2016). In US, in different backyard poultry farms, 27 cases of paratyphoid Salmonella enterica, with 12 of the paratyphoid Salmonella enterica infections were attributed as the cause of mortality and an additional 15 cases were detected on general Salmonella surveillance and were not associated with clinical signs (Cadmus et al., 2019). However, in Chile, some researchers highlighted the importance of breeding backyard poultry on the spreading of Salmonella serovars potentially hazardous to public health. In a study conducted by Alegría-Moran et al. (2017), different serovars were detected in backyard flocks which are linked to human and animal clinical outbreaks. Based on the results of the study of Trung et al. (2017), the majority of human non-typoidal S. enterica outbreaks is the result of foodborne infections or of person-to-person transmission and S. enterica infections may also be acquired by environmental and occupational exposure to infected animals. In 2018, in our laboratory, after a suspected outbreak of foodborne infection, S.E. was isolated in homemade sweets containing mascarpone cream, a typical Italian dessert made with raw eggs produced using backyard hen’s eggs (unpublished data). The aim of this study was to evaluate the seroprevalence for S.E. and S.T. in ornamental backyard hens raised in different Italian regions, associated with culture methods to detect Salmonella spp. in eggs produced by the tested flocks.

MATERIALS AND METHODS

Tested flocks: A total of 24 ornamental chicken farms located in 8 different Italian regions were included in this study. The poultry flocks selected were composed by less than 250 ornamentals pure breeds chickens, reared with free-range method, for beauty competitions or for meat and eggs self-consumption. These breeds maintain their reproductive activity from 3 to 7 years of age, were not subject to light or temperature conditioning and followed their biological reproductive cycle. Anyhow, the subjects that do not respect the breed standard are not suitable for beauty exhibitions and are intended to self-consumption, while eggs are incubated or used for home consumption or sold. Other avian ornamental species, such as waterfowl (goose and ducks), turkeys, guinea fowl, pigeons and peacocks were present in some farms, both multispecies and single species farms were considered. The main characteristics are summarised in Table 1.

Poultry feed: The feed was different for each farm, dry diet with cereals such as corn, oats, barley, wheat, sorghum, flour soya extract mix, or semi-solid, represented by mash, composed of cereal flours or by-products traditionally mixed with whey or warm water, vitamins and sunflower or soy oil. The animals were also fed with commercial feed in the first phases of growth from 0-80/90 days of age.

Vaccinations program and antibiotic treatments: The vaccination program for the main diseases of poultry of each tested flock is summarised in Table 1, no vaccination against S.E. and/or S.T. was performed and no antibiotics were administered in the 6 months preceding the sampling.

Sampling

Sample size: The total amount of hens in 24 farms was 1204 accounted for 75.39% on total of 1597 chickens. The hens included in the age-range between 5 month and 5 years, were 971 (80.64%). Based on the possibility of identifying a Salmonella infection with a prevalence of 5% and 95% of confidence level, a minimum of 58 subjects were sampled to identify at least one positive subject. The sampling size was increased to 240 blood samples.

Blood-serum samples: The study was conducted according to the veterinary clinical practices for no-experimental purposes, as mentioned in Article 2, paragraph 1, Letter b, of Legislative Decree No. 26/2014. With the voluntary consent of farmers, an aliquot of serum was used to verify the presence of S.E. and S.T. antibodies. Between December 2018 and February 2019, 10 blood samples (1.5 ml/sample) from 10 hens in reproductive state and asymptomatic, were obtained from each farm for a minimum percentage of 10% (Table 2). The serum obtained was frozen at -20°C until analysis.
21 eggs (Table 2) divided into 24 pools of egg yolk and shell were analysed.

**Table 1:** Location, poultry species, breeds reared and pathogens against which vaccination was applied in each tested farm

| Farm Regions | Poultry species and breeds | Vaccination |
|--------------|----------------------------|-------------|
| A Emilia-Romagna | Chickens (Robusta Lionata, Brahma, Orpington), Ducks, Goose, Peacock | ND, IB, FP, AE, IC |
| A1 Emilia-Romagna | Chickens (Polish, Silkie, Paduan, Brahma), Pigeons, Roulu Roulu | ND, IB, FP, IC, MD, MG |
| A2 Emilia-Romagna | Chickens (Faverolles, Cocin, Wyandotte) Ducks, Goose | ND |
| B Tuscany | Chickens (Leghorn) | ND, IB, FP, IC, MD, MG |
| B1 Tuscany | Chickens (Leghorn) | ND, IB |
| C Lombardy | Chickens (Sultan, Wyandotte, Orpington, Dwarf chickens, Leghorn, Sussex) | ND, IB, FP, IC, MD |
| C1 Lombardy | Chickens (Leghorn, Italener, Amburgo), Ducks, Goose, Turkeys | ND |
| C2 Lombardy | Chickens (Paduan, Polish) | ND |
| C3 Lombardy | Chickens (Barnevelder) | ND, IB, MD, ILT |
| D Piedmont | Chickens (Sicilian, Silkie, Cocin), Pheasant | ND |
| E Lazio | Chickens (Brahma, Cocin, Armock, Marans), Peacock | ND, IB, FP, IC, MD, MG |
| E1 Lazio | Chickens (Cemani, Lakenfelder, Orpington, Faverolles, Polish, Cocin, Vorwerk), Peacock, Ducks, Goose, Guinea fowl | ND |
| E2 Lazio | Chickens (Silkie, Chabo, Serama, Leghorn) | ND |
| E3 Lazio | Chickens (Paduan, Polish, Cornish), Turkeys, Pigeons | ND |
| E4 Lazio | Chickens (Silkie, Paduan, Polish, Cornish), Pigeons | ND |
| E5 Lazio | Chickens (Silkie, Cemani, Amburgo, Yokohama, Cornish, Marans) | ND |
| E6 Lazio | Chickens (Silkie, Australorp, Wyandotte, Araucana) | ND |
| G Trentino-Alto Adige | Chickens (Serama, Transilvania Naked Neck) | ND, IB, FP, IC, IB, MD, MG |
| H Veneto | Chickens (Silkie) | ND, MD |
| I Veneto | Chickens (Silkie) | ND, MD, ILT |
| I1 Veneto | Chickens (Silkie, Paduan, Polish) | ND, IB |
| I2 Veneto | Chickens (Polverara) | ND, FP |
| L Campania | Chickens (Ko-Shamo, Leghorn, Cornish, Wyandotte) | ND |

* A. ND (Newcastle Disease); IB (Infection Brochitis); FP (Fowlpox); AE (Avian Encephalomyelitis); IC (Infectious Coryza); MD (Infection Bursal disease); ILT (Infectious Larinatoechitis); MG (Mycoplasma gallisepticum).

**Table 2:** Sampling

| Farm Region | N° hens present in a farm/ N° total of chickens | N° hens in reproductive status/ N° total of hens | N° blood samples (%) | N° eggs sampled |
|-------------|-----------------------------------------------|-----------------------------------------------|----------------------|----------------|
| A Emilia-Romagna | 50/60 | 39/50 | 10 (25.64%) | 10 |
| A1 Emilia-Romagna | 100/120 | 68/100 | 10 (14.71%) | 10 |
| A2 Emilia-Romagna | 27/38 | 27/27 | 10 (37.04%) | 10 |
| B Tuscany | 70/100 | 53/70 | 10 (18.97%) | 10 |
| B1 Tuscany | 37/40 | 30/37 | 10 (33.33%) | 10 |
| C Lombardy | 93/130 | 63/93 | 10 (15.87%) | 10 |
| C1 Lombardy | 46/51 | 46/46 | 10 (21.73%) | 10 |
| C2 Lombardy | 60/75 | 60/60 | 10 (16.67%) | 10 |
| C3 Lombardy | 29/40 | 29/29 | 10 (34.48%) | 6 |
| D Piedmont | 48/75 | 48/48 | 10 (20.83%) | 10 |
| D1 Piedmont | 40/52 | 28/40 | 10 (35.71%) | 10 |
| E Lazio | 60/82 | 60/60 | 10 (16.67%) | 10 |
| E1 Lazio | 80/120 | 60/80 | 10 (16.67%) | 10 |
| E2 Lazio | 40/53 | 36/40 | 10 (27.70%) | 6 |
| E4 Lazio | 60/80 | 60/60 | 10 (16.67%) | 6 |
| E5 Lazio | 79/104 | 36/79 | 10 (27.78%) | 10 |
| E6 Lazio | 53/67 | 39/53 | 10 (25.64%) | 10 |
| E7 Lazio | 23/30 | 14/23 | 10 (71.43%) | 10 |
| G Trentino-Alto-Adige | 23/25 | 20/23 | 10 (50.00%) | 6 |
| H Veneto | 18/33 | 18/18 | 10 (55.56%) | 6 |
| I Veneto | 19/25 | 19/19 | 10 (52.63%) | 6 |
| I1 Veneto | 97/120 | 76/97 | 10 (13.16%) | 10 |
| I2 Veneto | 30/37 | 22/30 | 10 (45.45%) | 10 |
| L Campania | 22/40 | 22/22 | 10 (50.00%) | 10 |

**Table 3:** Serological results

| Farm Region | TSA-S.E. (Number Positive Samples/Total Samples) | TSA-S.T. (Number Positive Samples/Total Samples) | TSA-S.E. Inconclusive | TSA-S.T. Inconclusive | ELISA-S.E. | ELISA-S.T. | %Prev. S.E. (%) | Prev. S.T. (%) |
|-------------|-----------------------------------------------|-----------------------------------------------|----------------------|----------------------|------------|------------|----------------|----------------|
| A Emilia-Romagna | 0/10 | 0/10 | 0/10 | 1/10 | 1/10 | 0% | 10.0% |
| B Emilia-Romagna | 1/10 | 0/10 | 0/10 | 3/10 | 2/10 | 40.0% | 20.0% |
| E1 Emilia-Romagna | 0/8 | 0/8 | 0/8 | 3/8 | 1/8 | 12.5% | 23.0% |
| E2 Emilia-Romagna | 0/8 | 0/8 | 0/8 | 3/8 | 1/8 | 12.5% | 23.0% |

**Eggs samples:** From 6 to 10 eggs were taken for each group tested, kindly provided by the farmers who supported the study and stored at room temperature. Microbiological analysis was performed in pool for each farm, analysing yolk and shell pools separately. In total, 216 eggs (Table 2) divided into 24 pools of egg yolk and shell were analysed.

**Serological methods:** A Tube Serum Agglutination test (TSA) was performed according to Davies (2008) in O.I.E. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chap. 2.9.9., par. B.2.c.; 2018, Chap. 2.3.11, par. B.2.1.). Positive samples were tested using the following commercial kits ELISA: ELISA Kit IDEXX SE Ab X2™ (IDEXX Laboratories-Westbrook, Maine, US) for S.E.
and ELISA Kit X-Ovoflockscreen™ (x-OvO Limited, Canegie Campus, Dunfermline, UK) for S.T.

Microbiological methods: The culture methods on eggs, were performed according to UNI-EN-ISO. 6579-1:2017. According to the UNI-EN-ISO.6579-1:2017 procedure, the isolation of Salmonella (including S. typhi and S. paratyphi) from different matrices such as eggs (shell and yolk), feed, faeces e coacal swabs, is performed in two principal steps. In the first step, for each farm, yolk and shell pools were obtained from the eggs, and adequately homogenized in stomacher. Two hundred twenty-five ml of Buffered Peptone Water—BPW (Istituto Zooprofilattico della Lombardia e dell’Emilia Romagna, Brescia, Italy) at room temperature were added to 25 g of matrix (yolk and shell separately) and incubated at 36°C for 18±2 hours. In the second step, two selective- enrichment liquid media, Rappaport-Vassiliadis Soy - RVS (Istituto Zooprofilattico della Lombardia e dell’Emilia Romagna, Brescia, Italy) and Muller Kauamnn Tetrahtionate Novobiocin - MKTTn (Oxoid Deutschland GmbH, Wesel, Germany) were inoculated with 100 μl and 1.0 ml of culture broth respectively and incubated at 41.5°C and 37°C for 24±3 hours, respectively. In duplicate, a loop-full of broth was streaked on Xylose Lysine Desoxycholate - XLD (Oxoid Deutschland GmbH, Wesel, Germany) and Brilliant Green Agar - BGA medium (Meus s.r.l, Piove di Sacco, Padova, Italy) and incubated at 37°C for 24 hours. Colonies referable to Salmonella, appear pink with or without a black point in the center of the colony on XLD or BGA medium.

RESULTS

Serological results: From 971 ornamental hens, 240 (24.71%) blood samples (10 from each farm) were taken, 231 (24.01%) were analysed (Table 2). Nine serum samples (9/240, 3.75%) were rejected for insufficient quality. The percentage of animals sampled for each farm, in the age range considered, was between a minimum of 13.16% and a maximum of 71.43% (Table 2). In total, the positive farms were 4/24 (A-B-E1-E5) (16.67%) and the percentages of animals sampled in these farms were 25.64%, 18.87%, 16.67% and 27.78% respectively (Table 2). Based on the results obtained from serological tests in the positive farms (Table 3), the Salmonella serotypes detected and their prevalence were: in farm A, 1 sample resulted positive for S.T. (10.0% of prevalence); in farm B, 2 samples were positive for both serotypes and 2 samples for S.E. The prevalence for S.T. was 20.0% while for S.E. was 40.0%. In farm E1, 1 sample was positive for S.E. (12.5% of prevalence) and 2 samples for S.T. (25.0% of prevalence); in farm E5, 2 samples were positive for S.T. (25.0% of prevalence) (Table 3). Positive samples were in total 10/231 (4.33%). Out of 10 positive samples, 5/231 (2.16%) were positive for S.E., 7/231 (3.03%) for S.T., and 2/231 (0.87%) were positive for both serotypes (Table 3). About individual farms examined, 2/24 (8.33%) were positive for S.E. (B-E1) and 4/24 (16.67%) for S.T. (A-B-E1-E5). No farm resulted positive only for S.E. In 2 farms (H-L) TSA and ELISA serological test for S.T. had provided inconclusive results and the samples were excluded from the total count of the positive samples. In farms B and E1, positive for both serotypes, the prevalence was 40.0% and 12.5% for S.E., and 20.0% and 25.0% for S.T. respectively (Table 3).

Microbiological results: Microbiological analysis performed on 24 yolk pools and 24 shell pools were negative, with a Salmonella isolation rate of 0.0% (0/48).

Statistical analysis: Let’s consider the - farms where there are infected animals and that the spread rate is higher than 5% as predetermined (spread rate over the total population). Using the Cannon & Roe formula, the disease spread rate within a group can be detected with 95% confidence level depending on sample and group size. For example, by taking 10 blood samples from a farm represented by 40 hens, there is a 95% probability of detecting the presence of S.E. and S.T. in the group, if the spread rate is equal to or higher than 22%. In our study, out of 24 farms we have a rate of 15% in the farm with the lowest number of hens (farm E7, 14 hens) and a rate of 24% in the farm with the highest number of hens (11, 76 hens) (Table 2). Therefore, the collection of 10 blood samples is sufficient to detect the presence of S.E. and S.T., with 95% confidence level. In 4 positive farms (Table 3) the spread rate is 25%. It seems appropriate to use 10 blood samples as the number of samples to be taken, as it allows to find, with 95% confidence level, at least one infected subject. Paradoxically, in 2 farms out of 4 tested positive, positivity for S.E. and S.T. was found even with a number of samples analysed below 10 (farm E1 8/10; farm E5 8/10) (Table 3).

DISCUSSION

The present study detected 4/24 positive farms (16.67%), with a total of 10/231 samples positive for Salmonella spp. In particular, 2.16% for S.E. and 3.03% for S.T., with 2/231 (0.87%) samples positive for both serotypes, unlike was found in the study conducted by Brown et al. (2018) which detected a 0.0% serum prevalence for S.E. in a small flock of 41 backyard chickens. Brown et al. (2018) supports the extreme importance of the size of the sample, in order to obtain a valid and reliable epidemiological data. Our serological positivity, related to the size of the sample (231 blood samples), confirm that it is probably essential to increase the size of the sample when serological and epidemiological investigations are carried out, apart from what established by the preliminary statistical analysis. As a matter of fact if fewer animals would had been sampled, as 58 chickens, we would have probably underestimated the serological positivity, declaring farms as false-negative, when they were not. All pools of yolk and shell were negative for Salmonella spp. In culture method (0.0%). Is known that Salmonella spp. colonize the reproductive tissues, ovariates and oviducts, and it can survive inside the egg as well, in particular S.E. (Guard-Petter, 2001). Moreover, Salmonella spp. survives in the chicken endothelia reticulum, which has been demonstrated as an important host specificity, explaining their potential isolation in eggs (Foley et al., 2013). For these reasons, poultry can be persistent subclinical shedders, they appear healthy but can intermittently shed
bacteria and considering alternates eliminatory phases and latency phases (Behravesh et al., 2014), and become a possible reservoir of Salmonella. The negative result of isolation could be related with these factors. It should also be considered that the poultry investigated are not subject to particular productive and reproductive stress, opposed to intensive breeding. The negative result of the isolation of Salmonella from eggs could be also explained by the productive factors of the hens and correlated by age, in addition to the latency. Pure breeds produce less eggs than industrial laying hens. In our opinion, the number of eggs produced, a very variable age range of hens and production inconstancy, could affect the elimination of Salmonella in eggs. It must also be considered that backyard poultry owners have also limited access to specialized veterinarians and do not have the habit to investigate the causes of sudden death of their animals, which could result in a failure to detect a potential outbreak of Salmonella in early stages and consequently a failing in arresting the infection. Therefore, the negative results of the culture tests did not allow to investigate the genetic characterization of the isolates and verify their sensitivity to antibiotics. For further diagnostic investigation, to confirm the positivity of 10 hens, we could not obtain fecal samples and cloacal swabs, because farmers refused an additional assessment, probably fearing serious repercussions on their group of animals. After all, farmers are not subject to a legal obligation to conferred samples for official controls. However, we can state that the positive hens came into contact with S.E. and S.T. during their life, positivity had been confirmed also by ELISA test, and the present study suggest that S.E. and S.T. serotypes are the potential source of subclinical salmonellosis in ornamental poultry and the circulation of the pathogen raises a potential public health problem. The public health risk of foodborne infections, could increase with the trade of eggs produced by these hens, as permitted by Italian legislation. Human salmonellosis is most often of foodborne origin, but other routes of infection, such as contact with live animals and environmental transmission, have also been identified (Baker et al., 2007; O’Reilly et al., 2007). These animals are considered like a pet (McDonagh et al., 2018), and may inadvertently increase the risk of disease transmission, such as with only direct contact with feathers or beaks (Nichols et al., 2018). Based on the anamnestic data obtained in this study, the serum positivity for S.E. and S.T. found in the tested farms has not been associated with outbreaks of Salmonella infection of foodborne origin or linked to direct contact with live animals. The flocks examined were similar for zootechnical characteristics (free-range farms), chicken breeds reared (Mediterranean light chickens, Asiatic, Polish, French and American breeds) and presence of other ornamental avian species. The high serological prevalence for both serotypes of Salmonella spp. observed in two flocks with 12.5% and 40.0% for S.E., and 20.0% and 25.0% for S.T. respectively in B and E1 farm, compared with others 22 farms, could be explained by the higher chicken turnover in these two farms, a recent infection or recent participation to beauty competitions, where poultry come into contact with other subjects or the possibility of multiple interactions with other species. It should not be excluded exchanges of infected eggs between farmers may also take place. The presence of other avian ornamental species in these farms, conditions of biosecurity often not implemented (breeding of different species in separate paddock), general farm management, age and species of birds and exposure to infected birds/environments could have contributed to the observed difference in seroprevalence levels. The other avian species such as peacocks, pheasants, geese and ducks, have different reproductive cycles, oviposition does not occur in December or January, so it was not possible to analyze the eggs of these animals. Moreover, due to the excessive stress caused by the capture, it was decided to exclude these species from serological tests, as further sampling. Remains to investigate the role of these other avian species in the spread and maintenance of Salmonella spp. on farms. To our knowledge, this is the first investigation of seroprevalence for S.E. and S.T. associated to culture method on eggs in ornamental hens in Italy, and this study provides interesting preliminary information regarding the current prevalence of Salmonella in these types of farms. Anyway, raises many more questions regarding how this information fits in Italy current surveillance and monitoring of the disease, due the lacking about specific legislation. Due to our findings, we believe it is necessary to investigate further the circulation of zoonotic Salmonella species in these types of farms using a microbiological and biomolecular test, in order to have a clear view of its prevalence in backyard chicken flocks. Biosecurity practices of small poultry keepers are poor compared to commercial industries. Backyard chickens often have a regular contact with wild birds or mammals, or are often moved in promiscuous environment, for example for beauty competitions. We must also consider that the environmental persistence of Salmonella spp., high turnover of chickens and assiduous participation in beauty competitions, pose significant barriers to its elimination in the farms.

Conclusions: This study confirm that S.E. and S.T. are widespread in studied poultry farms as asymptomatic form. For this reason, it could be useful to inform farmers that a regular and periodic control of animals and eggs or meat that they consume or sell and limit direct contact with poultry (McDonagh et al., 2018), is very important to prevent and limit the spread of Salmonella foodborne infections, despite the inconsistency of current Italian legislation. Furthermore, the application of biosecurity standards is clear and simple for industrial breeders, but it is not so obvious that backyard farmers may be aware of the steps required to keep infectious diseases out of their flock and prevent their spread.

Authors contribution: AG and MF, conceived and designed the study. AG, GT and GM executed the experiments and PR and ER analysed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES
Abd El-fatah SS, Saad AS, Salam AESAAE, et al., 2020. Study on dispersal of Escherichia coli and Salmonella enterica in retail beef and chicken meat. Int J Vet Sci 9:309-12.
Alegria-Moran R, Rivera D, Toledo V, et al., 2007. First detection and characterization of Salmonella spp. in poultry and swine in backyard production systems in central Chile. Epidemiol Infect 135:3180-90.

Baker MG, Thormley CN, Lopez LD, et al., 2007. A recurring salmonellosis epidemic in New Zealand linked to contact with sheep. Epidemiol Infect 135:76-83.

Behravesh CB, Brinson D, Hopkins BA, et al., 2014. Backyard poultry flocks and salmonellosis: a recurring, yet preventable public health challenge. Clin Infect Dis 58:1432-8.

Brown JA, Bolts P, Marchi S, et al., 2018. Detection of Antibodies to Seven Priority Pathogens in Backyard Poultry in Trinidad, West Indies. Vet Sci 20: 5:11.

Cadmus KJ, Mete A, Harris M, et al., 2019. Causes of mortality in backyard poultry in eight states in the United States 31:318-26.

Davies R, 2008. Salmonellosis: OIE. Manual of diagnostic tests and vaccines for terrestrial animal (mammals, birds and bees) (6th ed.). Paris: Office International Des Epizootie. Chapter 2.9.9, p.1267.79.

EFSA, 2008. Scientific opinion of the panel on Biological Hazards on a request from EFSA on overview of methods for source attribution for human illness from food borne microbiological hazards. EFSA J 764:1-43. Retrieved from http://10.2903/j.efsa.2008.764.

EFSA, 2010. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2008. EFSA J 8:1496. Retrieved from https://10.2903/j.efsa.2010.1496.

EFSA, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J, 16:5500. Retrieved from https://doi.org/10.2903/j.efsa.2018.5500.

EFSA, 2019. Salmonella control in poultry flocks and its public health impact. EFSA J 17:5596. Retrieved from https://10.2903/j.efsa.2019.5596.

European Commission, 2003. Regulation of the European Parliament and of the Council of 17 November 2003, control of Salmonella and other specified food-borne zoonotic agents, 2160/2003/EC. In: Official Journal L 325/4, 12/12/2003.

European Commission, 2002. Regulation of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European food safety authority and laying down procedures in matters of food safety, 178/2002/EC. In: Official Journal, L 31, 01/02/2002.

European Commission, 2013. Regulation of the European Parliament and of the Council of 17 December 2013, establishing a common organization of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007, 1308/2013. In: Official Journal, L 347/818, 20/12/2013.

European Commission, 2007. Regulation of the European Parliament of the Council of 22 October 2007, establishing a common organization of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation), 1234/2007/EC. In: Official Journal, L 299, 16/11/2007.

European Commission, 2008. Regulation of the Commission Regulation of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs. 589/2008/EC. In: Official Journal, L 163/6, 24/06/2008.

Ferrari RG, Rosario DKA, Cunha-Neto A, et al., 2019. Worldwide Epidemiology of Salmonella Serovars in Animal-Based Foods: A Meta-analysis. Appl Environ Microbiol. 15; 85(14): e00591-19.

Foley SL, Johnson TJ, Ricke SC, et al., 2013. Salmonella pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev 77:582-607.

Guard-Petter J, 2001. The chicken, the egg and Salmonella Enteritidis. Environ Microbiol 3:421-30.

Italian Ministry of Health, 2013. Ministerial decree operating modalities of the computerized register of poultry farms, in implementation of article 4, of the legislative decree 25th January 2010, n. 9. (14A00354) (UG General Series n. 22 of 28-01-2014), 13.11.2013. In: Official Journal, n. 22, 28/01/2014.

Manning J, Gore V and Chousalkar K, 2015. Screening for Salmonella in backyard chickens. Prev Vet Med. 120:241-5.

McDonagh A, Leibler JH, Mukherjee J, et al., 2019. Frequent human-poultry interactions and low prevalence of Salmonella in backyard chicken flocks in Massachusetts. Zoonoses Public Health. 66:92-100.

Nichols M, Stevenson L, Whitlock L, et al., 2018. Preventing human salmonella infections resulting from live poultry contact through interventions at retail stores. J Agric Saf Health 31:55-66.

O’Reilly CE, Bowen AB, Perez NE, et al., 2007. A waterborne outbreak of gastroenteritis with multiple etiologies among resort island visitors and residents: Ohio, 2004. Clin Infect Dis 44:506-12.

Pohjola L, Nykäsenoja S, Kivistö R, et al., 2016. Zoonotic public health hazards in backyard chickens. Zoon Pub Health 63:420-20.

Trung NV, Carrrique-Mas JJ, Nghia NH, et al., 2017. Non-Typhoidal Salmonella colonization in chickens and humans in the mekong delta of Vietnam. Zoonoses Public Health 64:94-9.

UNI EN ISO 6579-1, 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part I: Detection of Salmonella spp. (ISO 6579-1:2017).

Vieira AR, Pires SM, Wegener H, et al., 2008. WHO Global Salm-Surv: Worldwide Salmonella distribution, 1995-2006. Abstract book of the International Conference of Emerging Infectious Diseases (ICEID), pp:167.

WHO: Salmonella, 2017. WHO. [Online]. [Accessed: 7 February 2019].