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Investigating *Salmonella* Serotypes Colonizing Laying Hen Housing Across Southern Greece: Implications to Public Health

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Abstract: In this research of *Salmonella* serotypes colonizing laying hen houses in Southern Greece, the facilities of 92 flocks were investigated by examination of feces and dust. Collected samples, 414 and 178 fecal and dust samples, respectively were cultured according to ISO 6579. The findings indicated that 64.1% of farm houses were found infected with one or more *Salmonella* serotypes. Of the samples examined, 36.5 and 51.1% fecal and dust samples respectively were found positive. Twenty different *Salmonella* serotypes were identified, amongst, which most prevalent were serotypes *Salmonella* enteritidis and *Salmonella* cerro. The proportion of facilities colonized indicated that salmonellosis of laying hens should be expected to be rather high in this area of Greece. Thus, mandating a wide and in depth investigation of food products' contamination and its relation to human recorded infections in Greece.

Key words: Eggs, laying, hen, poultry, public, Greece

INTRODUCTION

According to the report of the European Food Safety Authority (European Food Safety Authority, 2009) >50% of human salmonellos is due to eggs and egg products and 95% of them are caused by *Salmonella* enteritidis and typhimurium. *Salmonella* enteritidis, especially certain phage types are considered highly pathogenic for man and have been blamed for most human infections worldwide (Vugia *et al*., 2004; Van De Giessen *et al*., 2006; Rabsch *et al*., 2007). Open borders and free trade zones like the EU with many new member states lacking mechanisms for the systematic control of zoonotic agents are factors contributing to the spread of new *Salmonella* serotypes and phage types, considered pathogenic to man. Thus, many European nations have adopted measures to prevent contamination of the food production chain with *Salmonella* bacteria but with varying degrees of success (Van De Giessen *et al*., 2006; Namata *et al*., 2008).

Success in lowering the prevalence of infection to levels considered safe for consumers depends largely in recognizing the important risk factors, thus identifying the most effective measures for control (Mollenhorst *et al*., 2005; Van De Giessen *et al*., 2006; Namata *et al*., 2008). Control is currently mandatory for EU member states, which are obligated to enforce regulation (EU, 2160/2003, 1237/2007). In Greece, the reported to EFSA prevalence of salmonellosis in laying hen flocks was 49.3% for the year 2004-2005. This high prevalence indicates a difficulty in meeting reduction targets or avoiding without significant economic costs the restrictions set by EU regulations.

Thus, Greek laying hen farmers aware of the economic importance of these EU restrictions became keener in regularly testing their facilities and collaborate on measures to improve the health statues of their facilities hoping to avoid state control. Their attitude helped this research aiming to have an independent overview of the expected levels of infected flocks of laying hens, thus the possible threat to Public health. The research involved only flocks of laying hens located in Southern Greece and the islands.

MATERIALS AND METHODS

Laying hens: The housing facilities of 92 laying hen flocks of Southern Greece (islands included) were selected

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for investigating the spread of *Salmonella* serotypes. The selected method for investigating the status of a facility was the culturing of fecal and dust samples. Method of sample collection and amount of material collected was that described by Van De Giessen *et al.* (2006). Briefly, up to five pooled fecal samples and two dust samples per investigated house were transported in the laboratory in an ice box and tested within 48 h from collection.

Isolation of *Salmonella* sp.: The isolation of *Salmonella* sp. from collected samples was according ISO 6579 (ISO, 2002) with minimal modifications. Specifically, pre-enrichment was made using Buffered Peptone Water (BPW), enrichment was possible using the Modified Semisolid Rappaport-Vasiliadis media (MSRV) and final isolation of *Salmonella* was tried on XLD and RAMBACH solid media. All culture media were supplied by MERCK Co, (Athens, GR). Confirmation of the genus was possible using TSI, UREA, LYSINE and INDOLE biochemical reagents, while serotyping was performed at the National Reference Laboratory for Poultry Salmonellosis, Chalchida, Greece using Standard Laboratory Operating Procedures (SLOP) following the Kaufmann-White scheme.

RESULTS AND DISCUSSION

In total 414 fecal and 178, dust samples were tested deriving from the housing of 92 laying hen flocks. Fifty nine (64.1%) of the flocks examined were found having infected facilities with one or more serotypes of *Salmonella*. Of the 414 fecal samples 36.5% were positive. Of the 178 dust samples 51.1% were positive. The proportion of positive facilities by location was 61 in Peloponnesus, 63 in Sterea Hellas and 69% in the islands. The most often isolated serotypes of *Salmonella* were *Salmonella* enteritidis, *Salmonella* cerro and *Salmonella* livingston (Table 1). The three serotypes accounting for 55.3% of all identified serotypes.

The finding of the present wide investigation showed that the expected prevalence of *Salmonella* infected laying hen flocks should be much higher than that reported by EFSA for the year 2004-2005 in Greece. This very high proportion (64.1%) of infected houses is similar to that reported from some countries (Otoma *et al*., 2007) but lower to that reported from major European egg producers (Van De Giessen *et al*., 2006; Namata *et al*., 2008). *Salmonella* enteritidis was the most prevalent serotype (Table 1), accounting for 34% of all *Salmonella* serotypes identified but *Salmonella* typhimurium reported by EFSA as of equal importance was not among the most prevalent serotypes identified here. *Salmonella* enteritidis has emerged in recent years as one frequently isolated serotype of *Salmonella* sp. across the world from cases of human enteric disease (Vugia *et al*., 2004; Van De Giessen *et al*., 2006; Rabsch *et al*., 2007). Human salmonellosis in Greece was the most prevalent infection for the year 2008 and the findings here indicate that serotype *Salmonella* enteritidis should be expected to be the one most prevalent in humans. However, underreporting or reporting only cases requiring hospitalization could distort the importance of serotypes associated with human cases in Greece. In countries where human *Salmonella* infection is recorded in detail, the evidence shows that underreporting is not uncommon (Van Duynhoven *et al*., 2005; De Jong and Ekdahl, 2006). The same appears to be true in poultry (De Jong and Ekdahl, 2006), where the infection usually is not causing any clinical evidence. Thus, only the obligatory testing of all sources of *Salmonella* infections in man will show the extent of the problem across Greece.

As reported, a higher proportion of dust samples were found infected with *Salmonella* than fecal samples. Perhaps, this is reflecting the environmental conditions of hen houses e.g., method of cage cleaning or house ventilation. Dust could carry the bacteria from house to house or flock (Harbaugh *et al*., 2006) becoming an important risk factor for Greek flocks. Dust spreading should be expected during the dry summer months, a time that due to high temperatures the wide opening of windows is used to decrease indoor temperature conditions thus also increasing the risk for spreading the infection to neighboring flocks.

Although this investigation did not aim in identifying the important risk factors for Greek laying hen flocks in nations were salmonellosis is studied for many decades, flock size (larger) (Mollenhorst *et al*., 2005) and system of production (Namata *et al*., 2008) are the most important

Table 1: Number (N) of identified serotypes of *Salmonella* sp.

Serotype	N	Serotype	N	Serotype	N
Salmonella enteritidis	32	*Salmonella* typhimurium	4	*Salmonella* hadar	1
Salmonella cerro	12	*Salmonella* virchow	4	*Salmonella* blockley	1
Salmonella liningston	8	*Salmonella* mishmarhaemek	3	*Salmonella* cubana	1
Salmonella agona	4	*Salmonella* infantis	3	*Salmonella* heidelberg	1
Salmonella braenderup	4	*Salmonella* bredeney	2	*Salmonella* oranienburg	1
Salmonella rissen	4	*Salmonella* gallinarum	2	*Salmonella* worthington	1
Salmonella salamae	4	*Salmonella* albany	2		

risk factors. An additional risk factor is the mixing of ages in a production system or the increasing of the productive life of hens (Mollenhorst *et al*., 2005). Some or all of these risk factors are thought present among Greek flocks, thus possibly affecting the overall proportion of infected flocks and the serotypes identified. Thus, in Greece for successfully meeting (EU, 2160/2003, 1237/2007) currently in order a number of risk factors such as flock size, age of hens, production system, should also be investigated and eliminated. The high proportion of infected facilities indicate that for meeting the targets, infected flocks should be eliminated while high fines, in cases of re-infections could be required to keep flocks free of re-infection.

CONCLUSION

With this high proportion of infected flocks, one important step toward the control of sallmonela infection in laying hens is the adoption of measures identifiing and minimizing the risk factors. Additionally, vaccination appears as one good step toward consumer protection (Methner *et al*., 1997) but one should keep in mind that the available vaccines may not protect fully the public. This is demonstrated by the findings in Greece where only 36 of the 94 strains of *Salmonella* sp. isolate were of vaccinal serotypes (Table 1).

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