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Prevalence of *Salmonella* sp. Infections in Layer Flocks in East Azerbaijan Province of Iran

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Abstract: Salmonella is an important cause of human disease with an estimated 80.3 million annual foodborne cases and significant economical losses in the poultry sector worldwide. The aim of this study was to evaluation prevalence of Salmonella infections in layer flocks in East Azerbaijan province of Iran. For isolation of agent after autopsy of losses, liver surface was cauterized and swap from this area was obtained and added into the tetrathionate culture media and incubated at 37°C for 15 h. Then agents obtained from previous stage were cultured in the macconkey agar culture media as five regional method and then incubated at 37°C. After several times, lightly yellow colonies appeared that suggestive non-fermentative lactose bacteria. For differentiation of Salmonella from other non-fermentative lactose bacteria, specific Medias such as TST, urea, MR-VP, SIM, simmon citrate, lysine and Medias contain sucrose, lactose, maltose, manoz and arabinose used. Finally, the amounts of losses since week 31 until 67 was 5030 hens that by adding losses before week 31 which was about 1450, the sum of losses was 6480 or 27% of flock. This study revealed that contamination rate in East Azerbaijan province of Iran is higher than standard levels and because of zoonotical issues between poultries and human must be take measures in this field.

Key words: *Salmonella* sp. infection, layer flocks, macconkey agar, simmon citrate, Iran

INTRODUCTION

Salmonella is an important cause of human disease (EFSA, 2009; Newell *et al*., 2010) with an estimated 80.3 million annual foodborne cases (Majowicz *et al*., 2010) and significant economical losses in the poultry sector worldwide. In Europe, *Salmonella enteritidis* is one of the most commonly isolated serotypes in human salmonellosis (EFSA, 2007; WHO, 2006) where contaminated eggs produced by infected layers remain one of the most important sources of this infection (Crespo *et al*., 2005; Dejong and Ekdehl, 2006; Delmas *et al*., 2006; Korsgaard *et al*., 2009; Stevens *et al*., 2009). Today, the goal of layer producers is to ensure consumers have access to safe and wholesome egg products which necessitates initiation of control of this pathogen at farm level. For this aim in the country for example, most of the layer companies inform us that they use Salmonella vaccines against the most prevalent or endemic serovars in their regions, complemented with their biosecurity programs. These programs which are designed by the veterinarians of the breeder companies may cause problems in operational completion of prerequisite programs and Hazard Analysis and Critical Control Point Plans for the prevention of Salmonella contamination in flocks. Also, to evaluate the efficiency of these biosecurity actions including vaccinations these companies submit samples from their flocks to external diagnostic laboratories. Part of this control involves testing of samples collected by these companies for the presence of Salmonella with a traditional culture technique which requires 5-11 days for confirmation of the results. The most commonly applied and accepted gold standard culture method for the detection of *Salmonella* sp., in animal feces and in samples from the primary production stage is International Organization for Standardization (ISO) Method 6579:2002/ and 1:2007 in the countries of the European Union and this method also involves 4-6 days. To shorten this detection time in the interest of layer companies, realtime PCR (rPCR) can be applied to fecal samples before culture results (Eriksson and Aspan, 2007; Eyigor and Carli, 2003; Eyigor *et al*., 2002; Hadjinicolaou *et al*., 2009; Kurowski *et al*., 2002; Tomas *et al*., 2009). However as has been previously pointed out (Eyigor *et al*., 2002), the fact that rPCR and culture can complement each other and cannot replace one another should always be kept in mind.

In several countries, notably the UK, Spain and the US there has been a dramatic increase of Salmonella enteritidis infection in humans during the last 5-10 years.

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In England and Wales isolations of *S. enteritidis* from humans increased from 1087 in 1981-15427 (56% of a total of 27478 isolates of Salmonella) in 1989 whereas infections due to all other serovars combined increased by about half (Cowden *et al*., 1989; Frost *et al*., 1989). Similarly in Scotland, *S. enteritidis* infections in humans increased from 11% of all isolates of Salmonella in 1982-52% for the 1st 11 months of 1988 (Sharp, 1988). In Spain, outbreaks of food-borne disease in humans due to *S. enteritidis* increased from 8% in 1977 to 40% in 1984 whereas food borne disease due to *S. typhimurium* remained stable at 8%. In the US, reported *S. enteritidis* infections in humans increased from about 6% of all human isolates of Salmonella before 1976 to >51% in 1987 (Hopper and Mawer, 1988). In Canada, *S. enteritidis* was the third commonest isolate of Salmonella (8-3% of a total of 10646 isolates of Salmonella) from people in 1987 and the fourth commonest isolate (9-2% of 9957 isolates of Salmonella) in 1988. In Europe most human *S. enteritidis* isolates belong to Phage Type (PT) 4 (Arnold *et al*., 2010; Carrique-Mas *et al*., 2009). *Salmonella enteritidis* strains isolated in Canada and the US belong primarily to PT 8: 64% of the human isolates of *S. enteritidis* in Canada, 48% of human and animal and 64% of animal isolates of *S. enteritidis* in the US were phage type 8.

Outbreaks of disease by *S. enteritidis* in humans have been associated with the consumption of eggs or foods that contain eggs (Cowden *et al*., 1989a, b; Steinert *et al*., 1990). It has been suggested that *S. enteritidis* may infect eggs by transovarian transmission (Snoeyenbos *et al*., 1969; St Louis *et al*., 1988). The prevalence of isolation of *S. enteritidis* from hens and ovaries and eggs of hens varied considerably among different studies (Hopper and Mawer, 1988; Humphrey *et al*., 1989). Hopper and Mawer (1988) isolated *S. enteritidis* from 13 of 50 dead hens taken from a commercial layer flock of 60000 hens that was epidemiologically identified as the source of raw shell eggs that caused an outbreak of human food poisoning by *S. enteritidis* PT 4. *Salmonella enteritidis* was cultured from the fecal contents of 13, the oviduct of 8 and the ovaries of 6 of the 50 hens (Hopper and Mawer, 1988).

Examination of the egg contents of 1119 eggs derived from two small flocks of 12 and 23 egg-laying hens each showed that 11 eggs were positive for *S. enteritidis* (Humphrey *et al*., 1989). The production of infected eggs was clustered though intermittent. The positive eggs which were produced by 10 of the 35 hens all contained <10 Salmonellas (Humphrey *et al*., 1989). Examination of 15000 eggs derived from >1300 layer flocks in the US resulted in the detection of only one flock infected with *S. enteritidis*. Trace backs from infected people to three egg-producing flocks resulted in the isolation of *S. enteritidis* from yolk of eggs in one flock from a pooled ovarian sample of another flock and no isolations in the third flock. In orally infected and contact-exposed hens intestinal colonization persisted for as long as 18 weeks some strains of *S. enteritidis* caused significant decreases in egg production and *S. enteritidis* was found in a high percentage of the yolks and albumens of eggs laid during the 1st 2 weeks after inoculation. The aim of this study was to evaluation prevalence of Salmonella infections in layer flocks in East Azerbaijan province of Iran.

MATERIALS AND METHODS

In this study, diagnosis of Salmonella in hens was conducted as clinical signs and then for definitive diagnosis acting to culture of isolated genera from viscera and serologic examinations. For isolation of agent after autopsy of losses, liver surface was cauterized and swap from this area was obtained and added into the tetrathionate culture media and incubated at 37°C for 15 h. Then, agents obtained from previous stage were cultured in the macconkey agar culture media as five regional method and then incubated at 37°C. After several times, lightly yellow colonies appeared that suggestive non fermentative lactose bacteria. For differentiation of Salmonella from other non-fermentative lactose bacteria, specific Medias such as TST, urea, MR-VP, SIM, simmon citrate, lysine and Medias contain sucrose, lactose, maltose, manoz and arabinose used.

Finally, act to antibiogram by using of colonies obtained from tetrathionate culture media. These colonies were cultured in mueller hinton agar as uniform culture method and then specific antibiotics-dipped disks were located in plate. Plates were incubated at 37°C for 24 h. After these steps, *Salmonella enteritidis* approved.

RESULTS AND DISCUSSION

Finally, the amounts of losses since week 31 until 67 was 5030 hens that by adding losses before week 31 which was about 1450, the sum of losses was 6480 or 27% of flock (Table 1 and 2). In this study, researchers observed a considerably high Salmonella incidence in layer flocks because more than half of the samples submitted to the laboratory were determined to harbor this pathogen. There are similar previous findings from layer flocks in the percentages as high as 55.6, 76.9 and 86.5% by Dorn and Schleiff (1997), Carli *et al*. (2001) and

Table 1: Antibiotic results

Antibiotic	Resistance			
	Sensitive	Moderate	Resistant	Choice
Florfenicol	*	-	-	4+
Enrofloxacin	*	-	-	4+
Difloxacin	*	-	-	3+
Ampicillin	-	-	*	-
Erythromycin	-	-	*	-
Neomycin	*	-	-	2+
Oxytetracycline	-	-	*	-
Tetracycline	-	*	-	-
Trimethoprim	*	-	-	3+
Colistin	-	-	*	-
Tiamulin	-	-	*	-
Danofloxacin	*	-	-	1+

Table 2: Data related to losses associated to Salmonella by week

Weeks	Fri.	Sat.	Sun.	Mon.	Tue.	Wed.	Thu.	Total
31	35	43	35	28	62	67	55	325
32	51	62	48	35*	25*	18*	9*	248
33	4	5	7	3	4	5	2	30
34	5	5	4	3	3	4	2	28
35	4	4	3	2	5	7	5	30
36	52	48	48	45	58	53	50	354
37	57	58*	47*	38*	27*	18	9	254
38	3	7	10	6	4	2	6	38
39	9	4	6	5	2	7	8	41
40	4	4	4	3	7	6	7	35
41	7	7	9	3	21	36	17	100
42	29	41	47	53	68	72	45	355
43	53*	27*	31*	19*	17	13	11	171
44	12	9	11	7	4	8	9	60
45	7	11	12	9	6	4	3	52
46	3	6	2	8	11	7	6	43
47	33	47	42	63	62	53	56	356
48	54*	49*	36*	18*	9	11	8	185
49	7	5	6	8	6	4	2	38
50	2	6	7	6	5	2	4	32
51	13	18	38	47	53	56	68	293
52	54*	41*	32*	17*	16	9	3	172
53	14	11	3	2	6	7	4	47
54	6	4	8	3	7	2	6	36
55	3	2	5	2	7	11	9	39
56	11	27	43	49	63	47	53	283
57	67	58	53	63	43	36	52	372
58	49	43	28	14	7	3	8	152
59	3	2	6	4	7	3	2	27
60	3	3	3	6	4	2	5	26
61	11	4	6	2	5	6	3	37
62	2	1	4	3	6	2	9	27
63	17	13	22	27	42	36	11	168
64	29	53	64	18	64	51	71	350
65	49*	38*	29*	16*	7	11	4	154
66	9	3	4	6	4	3	2	31
67	6	3	7	2	9	11	3	41

*Indicates days of antibiotic administration

Li *et al.* (2007), respectively. There are also, studies reporting Salmonella detection rates as low as 0.0-17.0% (Ata and Aydin, 2008; Eyigor and Carli, 2003; Eyigor *et al.*, 2002, 2005; Kalender and Muz, 1999) from the country and 9.93-17.9% from European Union countries (Madden, 1989; Hoorebeke *et al.*, 2010). Apart from the effects of Salmonella prevalence within a flock (Arnold *et al.*, 2010) of the housing system (Carrique-Mas *et al.*, 2009; Huneau-Salaun *et al.*, 2009) and of the flock characteristics (Namata *et al.*, 2008), the variations in Salmonella detection rates have particularly been related to the sample type analyzed and the method used (Arnold *et al.*, 2010; Kinde *et al.*, 2005; Rybolt *et al.*, 2004).

Considering the fact that the environment of adult hens was examined, it was expected that the percentage would be lower because chickens rapidly become resistant to Salmonella infection with increase in age (Milner and Shaffer, 1952; Sadler *et al.*, 1969). The finding that such a high percentage of the environmental samples were contaminated with Salmonellas may be explained by persistence of contamination in poultry houses for long periods of time and for consecutive generations of birds. Snoeyenbos *et al.* (1970) noted that residual house contamination was frequent following depopulation cleaning and disinfection. Higgins *et al.* (1982) found that dust was contaminated with Salmonellas in 6 of 9 houses after disinfection and suggested that defects in the cleaning and disinfection of air inlets and fans seemed to be an important factor for recontamination of the house.

The isolation rates of Salmonella serovars from faecal and eggbelt samples appear to correlate. This suggests that when faeces are contaminated with a serovar also, the environment such as dust, feathers, shells, fluff and other debris will be contaminated with the same serovar and conversely when the environment is contaminated with a certain serovar, the hens may become infected and shed the same serovar in the faeces.

In this study by Poppe *et al.* (1991) revealed that overall rates of seropositivity of 248-299 sera from the seven positive flocks against either variant or standard strains of *S. pullorum* ranged from 29-77%. Substantially more birds were seropositive in flocks in which infected birds were subsequently identified (Poppe *et al.*, 1992).

CONCLUSION

In this study, overall incidence of Salmonella in layer flocks by rPCR and culture was 61.0 and 55.6%, respectively where 70.1% of these Salmonella isolates were determined as *Salmonella enteritidis*. Incidences of *Salmonella enteritidis* in culture-positive samples were 65.3% in cloacal swabs, 50.0% in intestines, 73.9% in gizzard swabs and 87.5% in cecal swabs. The rPCR results were in 100% agreement (100% sensitivity and specificity) with culture results when cecal swabs were selected as the sample type (Temelli *et al.*, 2010). Poppe *et al.* (1991) showed that the most prevalent serovars were

S. heidelberg, *S. infantis*, *S. hadar* and *S. schwarzengrund*, they were isolated from samples of 59/295 (20%), 18/295 (6-1%), 17/295 (5-8%) and 15/295 (541%) flocks, respectively. Feed samples of 21/295 (7-2%) flocks were contaminated with Salmonellas. *Salmonella enteritidis* was isolated from the environmental samples of 8/295 (2-7%) flocks (Temelli *et al*., 2010). By comparison of above mentioned results from other researchers revealed that contamination rate in East Azerbaijan province of Iran is higher than standard levels and because of zoonotical issues between poultries and human must be take measures in this field.

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