Prevalence and risk factors for *Listeria monocytogenes* contamination in Iranian broiler flocks

Saeed Seifi

ABSTRACT

Background: Microbiological safety and quality of broiler meat are equally important to producers, retailers and consumers. Listeriosis is one of the important emerging bacterial zoonotic infections worldwide. Among the different species of the genus *Listeria*, *Listeria monocytogenes* is known to cause listeriosis in humans and animals. *Listeria monocytogenes* is a causative agent of listeriosis, a severe foodborne disease associated with a high case fatality rate. Several food products have been linked with epidemics and sporadic cases of listeriosis, including meat, dairy, vegetable and fish products. To prevent product contamination with *L. monocytogenes*, it is essential to understand listerial contamination routes in the food processing industry. There are few published data concerning risk factors for *L. monocytogenes* contamination in poultry flocks. Information on the occurrence and distribution of *Listeria monocytogenes* and its risk factors is very limited both in the veterinary and public health sectors in Iran. The aim of this study was to investigate the prevalence and potential risk factors for *Listeria monocytogenes* contamination in Iranian broiler flocks.

Materials, Methods & Results: In this study, farms were separated in two different zones (foothill area in west with less humidity compared with coastal area in north with high humidity). The prevalence of listeriosis in coastal area was higher (52%) than foothill (48%) but not significantly (*P* = 0.642). A total of 1470 cloacal swab samples were randomly collected from 490 broiler flocks on the slaughter line of 4 abattoirs (2 abattoirs in north and 2 abattoirs in west) of Iran from March 2009 to December 2010. The PCR method with published primers targeting the hemolysin (*hly*) gene was used to detection of *Listeria* in cloacal swab samples. To evaluate potential risk factors, each flock was visited once. A questionnaire was completed for each flock. According to the results, the prevalence of *L. monocytogenes* in north was relatively higher (52%) than west (48%), however not significantly different. In addition, this work presented the relationships between *L. monocytogenes* and some risk factors.

Discussion: This is the first time that an epidemiological study of risk factors for *L. monocytogenes* contamination in broiler flocks was carried out in Iran. *L. monocytogenes* was isolated in 44 of 490 flocks; yielding an estimated prevalence of 8.97%. The prevalence of *L. monocytogenes* in coastal area was relatively higher (52%) than foothill area (48%). Higher prevalence of *Listeria* was observed in flocks that used private water sources in comparison with flock that used official approved water, but it was not significant. When pets were present on the flocks site the risk of *L. monocytogenes* contamination was increased. Disinfection of water and feed by commercial disinfectants was found to decrease the risk of contamination. No relation between a special strain with *L. monocytogenes* status could be found. In conclusion, this study has demonstrated the presence and distribution of *L. monocytogenes* in Iranian broiler flocks. The study also showed some potential risk factors that influence on prevalence of listeria in broiler flocks. It was proved that some relationships occur between *L. monocytogenes* prevalence and the flock’s litter quality.

Keywords: *Listeria monocytogenes*, prevalence, risk factors, listeriosis, broilers, Iran.
INTRODUCTION

It is well documented that the contamination of food with pathogens is a major public health concern worldwide. In the absence of hygienic conditions, the poultry may be highly exposed to bacterial pathogens such as *Listeria, Campylobacter*, and other enteric bacteria [9]. Among the different species of the genus *Listeria*, *L. monocytogenes* has been known to cause listeriosis which recognized as an important food-borne pathogen in many industrialized countries [23]. *Listeria monocytogenes* is a gram-positive bacterium that is widely distributed in the environment. Feces, soil, water, and decaying plant material are common sources for *Listeria* [13,28]. Various species of mammals and birds may be infected with *Listeria*, or they may serve as asymptomatic carriers [4,28].

Listeriosis is a relatively rare but serious disease with the highest hospitalization rate amongst known food-borne pathogens of 91% and a fatality rate ranging from 20 to 30% [24,32]. Outbreaks of listeriosis occur sporadically in chickens, turkeys, waterfowl, pigeons, and other avian species. Young birds are most susceptible [4].

The consumption of food contaminated by *L. monocytogenes* has been identified as the main transmission route for this pathogen in both humans and animals. *L. monocytogenes* has been isolated from all categories of food [10]. There are many reports about isolation and detection of *L. monocytogenes* from vegetables [1], milk and milk products [12,21], fish and seafood [11,15,25] and raw poultry meat [3,7,20,22,26]. Very few studies have reported an incidence of *L. monocytogenes* at the farm level [8]. The aim of this study was to investigate the prevalence and potential risk factors for *L. monocytogenes* contamination in poultry production.

MATERIALS AND METHODS

Collection of Samples

A total of 1470 cloacal swab samples were randomly collected from 490 broiler flocks (3 swabs per each flock) on the slaughter line of 4 abattoirs (2 abattoirs in north and 2 abattoirs in west) of Iran from March 2009 to December 2010.

Data collection

To evaluate potential risk factors, each flock was visited once. A questionnaire that structured the parameters such as: location, litter material and litter condition, drinker systems (Origin, Treatments) and feed/water treatment was completed for each flock.

Detection of Listeria

PCR primers targeting the hemolysin (*hly*) gene were used for confirmation as previously described [18]. Briefly, the reaction was done under the following thermocycling conditions; 60°C, 60 s; (annealing), 94°C, 60 s; (denaturing), 72°C, 60 s; (extension) for a total of 30 cycles. The sequences of the primers are shown in Table 1.

The templates were stored in 50/50 BHI/glycerol at -80°C until used. They were streaked onto TSAYE agar and colonies were swabbed into sterile water and lysed by boiling. The PCR reaction mixture contained the following concentrations of components for a total volume of 25 uL: 1X PCR buffer, 2.0 mM each dNTP, 2.0 mM MgCl2, the primers (F) 0.98 uM and (R) 0.74 uM and finally the enzyme Taq DNA polymerase 0.3 U/uL. The PCR products were visualized on a 1.5% agarose gel. The expected size of the PCR product was 234 bp.

Statistical analysis

The Chi-square and Fisher exact tests were used to determine significant differences between proportions. A probability of less than 0.05 was considered statistically significant.

RESULTS

Altogether 132 samples (8.97%) from the whole 1470 examined samples, were positive in PCR reaction. *L. monocytogenes* were detected in 44 of 490 flocks. The prevalence of *L. monocytogenes* in coastal area was relatively higher (52%) than foothill area (48%), however not significantly different. In addition, this work presented the relationships between *L. monocytogenes* and some risk factors (Table 2). As shown in Figure 1, amplification of an expected DNA bands from the positive control confirmed that the reaction has been performed correctly.
Table 1. PCR primers used for *L. monocytogenes* detection.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequence (5′→3′)</th>
<th>PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>hly</em></td>
<td>(F) CGGAGGTTCCGCAAAGATG</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td>(R) CCTCCAGAGTGATCGATGTT</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Relationships between *Listeria monocytogenes* infection and various risk factors.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of samples</th>
<th>Number of <em>L. monocytogenes</em> positive samples (%)</th>
<th>Chi-square ($\chi^2$)</th>
<th>df</th>
<th>Significance of Difference ($P$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foothill</td>
<td>112</td>
<td>4 (3.57)</td>
<td>0.323</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td>Costal</td>
<td>387</td>
<td>30 (7.75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Official</td>
<td>132</td>
<td>2 (1.51)</td>
<td>0.617</td>
<td>1</td>
<td>0.671</td>
</tr>
<tr>
<td>Well</td>
<td>358</td>
<td>29 (8.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of disinfectant in</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>115</td>
<td>8 (6.95)</td>
<td>0.086</td>
<td>1</td>
<td>0.672</td>
</tr>
<tr>
<td>No</td>
<td>375</td>
<td>25 (6.66)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of disinfectant in</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>feed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>100</td>
<td>5 (5)</td>
<td>1.882</td>
<td>1</td>
<td>0.339</td>
</tr>
<tr>
<td>No</td>
<td>390</td>
<td>34 (8.71)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moist</td>
<td>59</td>
<td>30 (50.8)</td>
<td>8.23</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Dry</td>
<td>431</td>
<td>14 (3.24)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Figure 1. Agarose gel electrophoresis of *L. monocytogenes* amplification products (*hly* PCR). M: DNA marker (100 bp), L1: positive control, L2: negative control, L3-5: negative samples, L6-7: positive samples.
DISCUSSION

This is the first time that an epidemiological study of risk factors for *L. monocytogenes* contamination in broiler flocks was carried out in Iran. *L. monocytogenes* was isolated in 44 of 490 flocks; yielding an estimated prevalence of 8.97%. *Listeria monocytogenes* was the most common bacterial contaminant on broiler chickens at slaughterhouses [19]. Some research were conducted to evaluate the prevalence of *Listeria* in poultry population in the different zone of the world, such as the work of Chemaly et al. [8] that reported 15.5% and 32% contamination by *L. monocytogenes* from laying hens and broilers, respectively. Another study reported that *L. monocytogenes* was not detected from the cloacal swabs taken from the chickens entering slaughterhouses, but was detected from 59 (2.5%) out of 2,327 samples of frozen chicken meat [16]. In present study, the low detection rate for *L. monocytogenes* on broiler samples may interoper ite use of antibiotics that given to broilers during rearing period. There are many researches about detection of *Listeria* in raw poultry meat. Kosek-Paszkowska et al. [17] reported that *Listeria species* were present in 36 samples of raw chicken parts (51.4%) and in 7 samples of poultry minced meat (30.4%), and the prevalence of *L. monocytogenes* was 14%. The considerably higher level of contamination of poultry raw meat from supermarkets in Spain [6]. *Listeria* was found in as much as 95% of examined carcasses and 32% of them were recognized as *L. monocytogenes*. Vitas et al. [27], reported 36.1% positive samples of raw poultry in their survey car ried out in Northern Spain. Similar researches were reported from Finland, Belgium, Nordic countries and Japan [17]. Broiler flock drinking water sources were noticed in this study, and according to the results the higher prevalence of *Listeria* was observed in flocks that used private water sources (unofficial water supply such as wells) in comparison with flock that used official approved water, but it is not significant (P = 0.671). The density of broilers in each flock were analyzed, but there is not a significant relation between overcrowded flock and prevalence of *Listeria* (P = 0.845). The relation between type of flock litter (i.e., straw, wood shavings) and prevalence of listeria were studied. The must used litter was straw, but there was not significant relationship (P = 0.385). Moisture of the litter was significantly influence on prevalence of *Listeria* (P = 0.03). This finding was supported by the previous investigation [2]. Survival of *L. monocytogenes* in soil depends on the soil type and its moisture content and humid organic material favors *L. monocytogenes* growth [30]. The relationship between presence of dog in the flock site and *Listeria* was investigated. Some of farmers like to presence of one or more dog in own poultry flock site for guard against the thieves. The presence of dogs on the poultry flock site was found to increase the risk of *L. monocytogenes* contamination in flocks. *L. monocytogenes* is commonly found in the excrement of a large number of animal species [28], but few studies report the incidence of *L. monocytogenes* in pets [29]. Dogs can then be vectors and play a role in the multiplication of *Listeria* by excreting a great number into the environment. A study carried out in broiler flocks showed that pets around the poultry house (within 5-10 m) associated with the absence of sanitary barriers at the poultry house entrance, was significantly associated with an increased risk of *Campylobacter* contamination [14]. It was reported that the presence of pets on the production site was found to increase the risk of *L. monocytogenes* contamination in laying hen flocks [2].

According to use of disinfectants in feed meal and drinking water, flocks separated into two groups. Results showed that the prevalence of *L. monocytogenes* in flocks that use a kind of disinfectant in feed meal had lower contamination with *Listeria*, significantly (P = 0.045). This finding is in line with previous study [2]. Feed can play a role in the transmission of *Listeria*, which has been found in poultry in pellet form [5,31]. It seems that feed meal disinfection have a protective effect against *L. monocytogenes* contamination in broiler chickens. There was not a significant relationship between drinking water disinfection and prevalence of *Listeria* (P = 0.854).

Occurrence rate of *Listeria* in different commercial strains (Ross, Cobb and Arbor Acres) was evaluated. No relation between a special strain with *L. monocytogenes* status could be found (P = 0.288).

In our study, farms were separated in two different zones (foothill area in west with less humidity compared with coastal area in north with high humidity). The prevalence of listeriosis in coastal area was higher (52%) than foothill (48%) but not significantly (P = 0.642).
CONCLUSION

In conclusion, this study has demonstrated the presence and distribution of L. monocytogenes in Iranian broiler flocks. The study also showed some potential risk factors that influence on prevalence of listeria in broiler flocks. It was proved that some relationships occur between L. monocytogenes prevalence and the flock’s litter quality. Future studies on the current topic are therefore recommended.

REFERENCES


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