Emergence of *Salmonella* Enteritidis outbreaks in broiler chickens in the Lebanon: epidemiological markers and competitive exclusion control


(1) Animal Science Department, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon
(2) Biology Department, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon
(3) HAWARCO, Poultry Research Department, Safra, Lebanon
(4) Department of Diagnostic Medicine, College of Veterinary Medicine, University of Minnesota, St Paul, 55108 Minnesota, United States of America
(5) SPARBOE, Commercial Poultry Layers, Litchfield, 55219 Minnesota, United States of America

This paper discusses the organism which for many years has been known as *Salmonella enteritidis* and which is now correctly known as *Salmonella enterica* subsp. *enterica* serovar Enteritidis, but for brevity is now called *Salmonella* Enteritidis.

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Summary

This study investigates the first emergence of *Salmonella* Enteritidis outbreaks among chickens in the Lebanon and identifies the epidemiological markers of selected recovered Enteritidis strains. In addition, the authors evaluate a competitive exclusion approach to control infection in broiler chickens by Enteritidis organisms which possess the prevalent identified markers.

The basic procedure in this investigation involved recording signs and lesions in eleven broiler chicken flocks on eleven farms, and culturing livers, spleens, and caeca of ten randomly selected birds per flock for *Salmonella* isolation and serotyping. Furthermore, culturing for *Salmonella* and serotyping was attempted from the livers, spleens, caeca and oviduct swabs of ten hens in four broiler breeder flocks which provided hatching eggs for the broilers under study. The identification of epidemiological markers in recovered *S.* Enteritidis included the determination of drug-resistance patterns and plasmid profiling. The competitive exclusion was evaluated by spraying the microflora on day-old broilers in the hatchery, followed by a controlled oral challenge at three days of age, with $2.85 \times 10^5$ colony-forming units of *S.* Enteritidis organisms per bird. Exclusion was evaluated by culturing for *S.* Enteritidis in anal swabs, spleens, livers, and caeca of individual challenged birds treated with the microflora and in untreated challenged birds.

A total of 112 invasive *S.* Enteritidis strains were recovered on eleven farms from individual organs of broiler chickens with typical signs and lesions of salmonellosis. The prevalent resistance to drugs in such strains was to furaltadone and gentamycin, a marker identified in 93 strains (83%), recovered from nine out of eleven farms. The same resistance pattern was present in *S.* Enteritidis strains recovered from breeders on one out of four farms. The prevalent plasmid profile in nine *S.* Enteritidis organisms selected randomly from a pool of 93 strains (one per each of the nine broiler farms) was 14.1 kilobases (kb) and ~50.0 kb, a typical pattern to that identified in *S.* Enteritidis organisms recovered from oviducts of breeders on one out of four breeder farms. The exclusion significantly reduced cumulative mortality in birds of up to 45 days of age by 3.93%, in comparison to that observed in untreated challenged birds.
Rev. sci. tech. Off. int. Epiz., 18 (3) 711 (P < 0.05). At 45 days of age, exclusion resulted in a 15.6% reduction in the percentage infection rate by S. Enteritidis in spleens or livers and a 34.4% reduction in the percentage infection rate of the caeca (P<0.05).

Keywords

Introduction
The bacterium Salmonella Enteritidis has been associated with disease in broiler breeding stock by vertical transmission to progeny (25). Certain strains of Salmonella Enteritidis have been invasive in young chicks, resulting in high mortality in broiler flocks (2, 21). Consumption of eggs or broiler meat contaminated with S. Enteritidis has led to several major outbreaks of food-borne disease in humans over the last few years and to increased awareness of poultry industry and public health agencies to the high risk of infection by S. Enteritidis (12, 29). In the United States of America (USA), S. Enteritidis has emerged as the major aetiological agent of human salmonellosis resulting in two to four million cases each year (13).

Different markers have been used to study the relationship between S. Enteritidis strains recovered during epidemiological investigations of salmonellosis outbreaks in chickens and humans (3, 8, 27). Phenotypic markers included antimicrobial susceptibility testing (27), serotyping (3), colicin typing (38), and phage typing (4, 40). Some workers supplemented identification of the phenotypic markers in S. Enteritidis strains by plasmid profiling analysis (9, 33), leading to better establishment of a 'cause and effect' relationship (30).

It has been shown that the presence of high molecular weight (HMW) plasmids (approximately 50 kilobases [kb] or 36 megadaltons) in S. Enteritidis organisms leads to higher expression of virulence manifested in the systematic invasiveness of such strains (19, 20, 22, 26). Thus, identification of the presence of virulence associated-HMW (VA-HMW) plasmids in S. Enteritidis strains is becoming an indispensable task in investigations and evaluation of future control programmes of severe S. Enteritidis outbreaks in poultry, with the aim of reducing the transmission of such organisms to humans.

Research has recently focused on the development of microbiological prophylactic strategies in an attempt to prevent or control intestinal and tissue colonisation in chickens by invasive S. Enteritidis strains (35, 39). This approach, known as the 'Nurmi concept' or 'competitive exclusion' (28), has opened new horizons for the control of Salmonella in poultry (36).

The aim of the present study is to investigate the first emergence of invasive S. Enteritidis outbreaks on major broiler chicken farms in the Lebanon. Epidemiological markers of selected S. Enteritidis strains recovered from broilers and broiler breeders are identified, including drug resistance and plasmid profile. In addition, a competitive exclusion approach is evaluated in an attempt to reduce infectivity in broilers by the most prevalent S. Enteritidis strain with predominant epidemiological markers.

Materials and methods
Investigation of Salmonella Enteritidis outbreaks
Eleven broiler chicken farms were included in an investigation of severe salmonellosis outbreaks occurring between the ages of eight and fifteen days. Mortality and clinical signs were recorded during the visits to the eleven farms.

The average size of the flocks investigated on the eleven broiler farms was approximately 7,000 birds. Ten dead birds were randomly selected from each flock for autopsy, recording of lesions, and culturing for Salmonella isolation. The culturing was performed on the livers, spleens and caeca of the selected broilers. Four broiler breeder flocks, the source of day-old chicks to the eleven broiler farms, were also included in this investigation. Salmonella culture was attempted from livers, spleens, caeca and oviducts of ten individual breeders selected randomly from emaciated and weak parents in each of the four breeder flocks.

Salmonella isolation and typing
Culturing for Salmonella isolation was performed according to the methods described by the American Association of Avian Pathologists (AAAP) (1). Briefly, livers, spleens and caeca were each cut aseptically to an approximate weight of 1 g and cultured on tetrathionate broth for 24 h at 37°C. Oviducts of breeders were swabbed from inside to a distance of approximately 5 cm, using a sterile cotton swab, and then cultured similarly. Subculture from the tetrathionate broth was performed using a sterile loop on brilliant green agar plates. Colonies suspected of being infected with Salmonella were subcultured on triple sugar iron (TSI) agar slants and urea agar plates for 24 h at 37°C. Salmonella typing was performed on cultures with alkaline slants and acid bottoms producing H2S in TSI and with absence of urease production,
using specific antisera. Cultures identified with typical somatic and flagellar antigens conforming with S. Enteritidis included in the Kauffman-White scheme for Salmonella serotypes (17), were subjected to antimicrobial susceptibility testing, to determine the drug-resistance pattern.

Drug resistance pattern

The single disc diffusion method was used to determine the antimicrobial susceptibility of the confirmed S. Enteritidis strains (7). S. Enteritidis strains were each grown on brain heart infusion broth for a period of 6 h to 8 h at 37°C, and the density of the cells was adjusted by sterile saline to match the turbidity of 0.005% barium chloride in 0.36 N H₂SO₄. The adjusted culture was spread on Muller-Hinton agar plates using sterile cotton swabs, and eight antimicrobial discs, each with a potency of 30 µg per disc, were applied on the Muller-Hinton plates for 24 h at 37°C. The antimicrobial agents with their bracketed codes were as follows: amoxicillin (Amo), ampicillin (Amp), chloramphenicol (Chl), ciprofloxacin (Cip), colistine sulphate-doxycycline (Col), enrofloxacin (Enr), furaltadone (Fur) and gentamycin (Gm). Isolates with an inhibition zone diameter greater than or equal to 20 mm were considered sensitive to the antimicrobial agents, while those with zones of 0 mm diameter were considered resistant.

Plasmid isolation and profiling

Strains of S. Enteritidis recovered from broiler chickens which showed the same drug-resistance pattern were put in the same category. Approximately 10% of S. Enteritidis strains were randomly selected from each category for gel electrophoresis of their plasmid(s); however, all the S. Enteritidis strains recovered from broiler breeders were subjected to plasmid analysis, due to their limited number (Table I). Plasmid isolation was performed in accordance with a procedure described by Dorn et al., with minor modifications (16). In brief, the S. Enteritidis cells were lysed by 5 mg/ml of TGE buffer (25 mM Tris, 50 mM glucose, 10 mM EDTA [ethylenediamine tetra-acetic acid], pH 8) at 37°C for 15 min, followed by treatment with sodium dodecyl sulphate (1%) in 0.2 M NaOH. The cells were treated with 3 M of sodium acetate (pH 4.8) prior to centrifugation at 12,000 g. The plasmid DNA was extracted from the supernatant by a phenol-chloroform mixture (1.2 v/v) and then precipitated by absolute ethanol at -20°C. The pelleted plasmid was resuspended in 30 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8) supplemented with 5 µl of RNAase (1 mg/ml). Plasmid profiles were established by electrophoretic analysis on 0.6% agarose gels in TBE buffer (89 mM Tris base, 89 mM boric acid and 2.5 mM EDTA). The reference plasmid markers used were bacteriophage lambda DNA, single cut mixture of 1.1-48.5 kb range, and lambda DNA Hind III digest of 0.123 kb-23.1 kb. A standard curve was developed using molecular weights of the reference plasmid markers and the distance each migrated in the gel. The molecular weight of each plasmid in S. Enteritidis strains was observed in relation to lambda DNA markers and under the protocol conditions of this procedure.

Competitive exclusion study

One hundred and sixty-day-old broiler chicks of the same breeder flock, which were free of S. Enteritidis, were included in this study. Eighty-day-old birds were treated by spraying with a microflora claimed by the manufacturer to compete against Salmonella in poultry (28). The competitive exclusion microflora (CEM) was administered according to the instructions supplied by the manufacturer. The remaining 80 birds were left as untreated controls. The 80 CEM-treated birds were distributed evenly in two separate isolation rooms, and the untreated birds were treated similarly. At three days of age, all birds were challenged with the most prevalent S. Enteritidis strain recovered from a liver of one of the investigated broilers, one which had the most frequent plasmid profile of 14.1 and ~50 kb, and a predominant drug-resistance pattern of Fur-Gm. The challenge was

Table I
Characterisation of drug resistance patterns and plasmid profile markers useful in the epidemiology of Salmonella Enteritidis infection in broiler chickens

<table>
<thead>
<tr>
<th>Farms</th>
<th>S. Enteritidis isolation (No. of positive farms/No. tested)</th>
<th>No. of organs with S. Enteritidis Recovery/No. tested (%)</th>
<th>S. Enteritidis drug resistance pattern/No. of isolates/No. of farms</th>
<th>No. of randomly selected S. Enteritidis isolates for plasmid profile/No. of farms</th>
<th>Plasmid profiles of selected S. Enteritidis isolates (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>11/11</td>
<td>Liver: 22/110 (20%) Spleen: 33/110 (30%) Caecum: 52/110 (51.8%) Oviduct: NA</td>
<td>Col-Fur-Gm/9/1</td>
<td>1/1</td>
<td>1.8, 14.1, ~50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeders</td>
<td>1/4</td>
<td>Liver: 0/40 (0.0%) Spleen: 0/40 (0.0%) Caecum: 0/40 (0.0%) Oviduct: 4/40 (10.0%)</td>
<td>Fur-Gm/4/1</td>
<td>4/1</td>
<td>14.1, ~50</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

NA: not applicable

a) Ten birds per farm were cultured for Salmonella Enteritidis
b) Susceptibility of Salmonella Enteritidis organisms was tested in vitro using the single disc diffusion method, against discs of 30 µg potency of each of the following antimicrobial agents: amoxicillin trihydrate (Amo), ampicillin (Amp), chloramphenicol (Chl), ciprofloxacin (Cip), colistine sulphate-doxycycline (Col), enrofloxacin (Enr), furaltadone (Fur) and gentamycin (Gm)
administered orally to each bird in a dose of $2.85 \times 10^9$ colony-forming units (cfu)/ml. In each of the two treatments, the mortality in birds up to 45 days of age was recorded. Individual rectal swabs were collected for the culturing of Salmonella at 7, 10, 17 and 24 days of age, using the AAAP procedure described previously (1). At 45 days of age, spleens, livers and caeca of 32 birds per treatment, were cultured individually for Salmonella isolation as described previously.

**Statistical analysis**

The $\chi^2$ test was used to compare mortalities and frequencies of birds with S. Enteritidis infection in CEM-treated versus untreated birds, using a computerised statistical program called ‘M. Stat’ from Michigan State University in East Lansing.

**Results**

**Salmonella Enteritidis outbreaks and epidemiological markers**

The average cumulative mortality percentage in the eleven investigated broiler flocks, during the period from 8 to 15 days of age, was 4%. The predominant signs in the eleven flocks included somnolence, profuse diarrhoea followed by dehydration, pasting vents, drooping wings, shivering and huddling near the gas heaters. Table I shows the recovery of S. Enteritidis organisms from birds in the eleven investigated farms, with maximum recovery from the caecum (51.8%), followed by the spleen (30%) and liver (20%). The same Table displays the most common drug resistance pattern (Fur-Gm) present in 93 of 110 (84.5%) S. Enteritidis strains recovered from broilers on nine of the eleven farms investigated. The same drug resistance pattern was present in all S. Enteritidis strains recovered from oviducts of breeders present on one of the four breeder farms investigated. All S. Enteritidis strains recovered from breeders and breeders with this common drug resistance pattern (Fur-Gm) also had the same plasmid profile of 14.1 and ~50 kb as shown in Table I. Two other drug resistance patterns of similar low prevalence in S. Enteritidis strains recovered from broilers were Col-Fur-Gm (9 out of 110 strains, i.e. 8.2%) and Fur (10 out of 110 strains, i.e. 9.1%). Such drug resistance patterns were not encountered in S. Enteritidis strains recovered from breeders. In addition, this difference in drug resistance pattern was associated with a difference in plasmid profile (Table I).

**Competitive exclusion study**

The cumulative mortality up to 45 days of age in the CEM-treated and challenged broilers was 5.04% in comparison to the untreated challenged birds (8.97%) (Table II). The anal shedding of S. Enteritidis organisms at 7 and 10 days of age was similar in both treatments. However, the shedding of S. Enteritidis organisms was significantly reduced in the CEM-treated birds in comparison to untreated challenged birds at 17 days of age (18.18% versus 36% respectively, $P < 0.05$) and at 24 days of age (16.88% versus 35.16% respectively, $P < 0.05$). At 45 days of age, no S. Enteritidis were detected in the internal organs, spleens or livers of the CEM-treated birds, whilst the presence of S. Enteritidis was detected in 15.6% of the spleens or livers of the untreated challenged birds ($P > 0.05$). The CEM treatment was not able to prevent the colonisation of S. Enteritidis organisms in the caeca: 25% of caeca were colonised in comparison to 59.4% of caeca in the untreated challenged birds ($P < 0.05$).

**Conclusion**

The predominant signs described in the eleven broiler flocks, associated with a high average mortality of 4% between 8 and 15 days of age, and the high recovery of S. Enteritidis organisms from the internal organs (Table I) were indicative of the invasive nature of such organisms, resulting in severe salmonellosis. Previous surveillance of the occurrence of salmonellosis between 1992 and 1996 succeeded in recovering S. Enteritidis organisms only from commercial layers in the Lebanon (5); thus, the data presented in this paper describe the emergence of S. Enteritidis outbreaks in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality up to 45 days of age (%)</th>
<th>Frequency of anal shedding of S. Enteritidis (%)/No. tested at different ages</th>
<th>Frequency of organs infected with S. Enteritidis at market age, 45 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 days</td>
<td>10 days</td>
</tr>
<tr>
<td>CEM-treated</td>
<td>5.94 a,b</td>
<td>27/80 b</td>
<td>41/80 b</td>
</tr>
<tr>
<td>Untreated</td>
<td>8.98 b</td>
<td>31/79 b</td>
<td>50/79 b</td>
</tr>
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</table>

| CEM: competitive exclusion microflora

a) Three day old birds were each challenged in the mouth by $2.85 \times 10^9$ colony-forming units (cfu) of Salmonella Enteritidis carrying 14.1 and ~50 kb plasmids
b) CEM was sprayed at one day of age, according to the instructions of the manufacturer
c) *c-f* Frequencies in a column followed by different superscripts differ significantly ($P<0.05$)
broiler chickens in the Lebanon for the first time. This epidemiological information is becoming increasingly important during the new era of peace in the Middle East which has opened many borders for trade in poultry products among neighbouring countries, including Israel.

The presence of one plasmid (14.1 kb) in S. Enteritidis was associated with resistance to furaltadone (Table I); however, the presence of an additional ~50 kb plasmid increased the range of resistance to include gentamycin (Table I). Thus, the resistance to gentamycin is probably mediated by the nucleotide sequence in the ~50 kb plasmid. Moreover, the addition of a third plasmid (1.8 kb) increased the resistance range more significantly to include colistine sulphate-doxycycline (Table I). Thus, the resistance to colistine sulphate-doxycycline appears to be coded in the 1.8 kb plasmid. This concurs with work conducted by other research teams which has demonstrated that drug resistance is plasmid-mediated (24, 27).

The recovered S. Enteritidis strains from birds were placed into three categories, based on drug resistance patterns (Table I). The majority of the strains recovered from broilers and all strains recovered from oviducts of breeders fell into the Fur-Gm drug resistance category. All selected strains in this category possessed the same two plasmids, namely, the 14.1 kb and ~50 kb, leading to a 100% correlation between resistance pattern and plasmid profile. Such highly correlated epidemiological markers increased the confirmatory identity of the 'cause and effect' relationship, rendering the breeders the most likely source of transmission of S. Enteritidis organisms to offspring. However, additional molecular techniques are required for further confirmation. Reports from other parts of the world state that the occurrence of ovarian infection in some broiler breeders results in vertical transmission of S. Enteritidis organisms to offspring. However, additional molecular techniques are required for further confirmation. Reports from other parts of the world state that the occurrence of ovarian infection in some broiler breeders results in vertical transmission of S. Enteritidis organisms to offspring (25).

Fourteen of the fifteen S. Enteritidis strains contained the HMW plasmid of ~50 kb (~38 MDa) (Table I). This ~50 kb HMW plasmid is associated with virulence in S. Enteritidis infections (20, 26, 37). A plasmid of similar size was recently identified in most S. Enteritidis isolates of a variety of phage types recovered from poultry and poultry houses in many parts of the USA (9, 34). In addition, the VA-HMW plasmids are present in S. Enteritidis organisms of phage type 4 (14), recovered from chickens for the first time in the United Kingdom, resulting in severe damage to the British poultry industry and to serious outbreaks in the human population (6, 15). Future investigation will reveal the relationship between S. Enteritidis isolates recovered from the population in the Lebanon and those identified in chickens.

The CEM treatment of day-old broilers resulted in reduced mortality in comparison to untreated birds challenged at three days of age (Table II). This drop in mortality was associated with a reduction in frequency of birds shedding the S. Enteritidis organisms at 17 and 24 days of age, a reduction of colonisation in the caecum at 45 days of age and absence of detection of such organisms in the spleen and liver of treated birds. This indicates that CEM was able to reduce the intestinal colonisation, which probably reduced the probability of invasion by virulent strains of S. Enteritidis. Previous reports confirm the data obtained (32, 35, 39). More specifically, provision of CEM to broiler chicks effectively decreased the caecal colonisation by a number of Salmonella species (23, 36). The microflora applied in this experiment and a similar preparation (31) have been used successfully for over ten years in Sweden and Finland for protection against other mildly virulent paratyphoids infecting nearly 70% of broilers in these countries. The efficacy of this microflora against virulent S. Enteritidis phage type 4 was recently demonstrated in laboratory-scale experiments in the Netherlands (10) and the United Kingdom (11). On the other hand, a failure in the competitive exclusion effect of the microflora on Salmonella was reported in a field experiment from Switzerland (18). The contradiction in results from different parts of the world requires further investigations to study the competitive exclusion effects of microflora against VA-HMW plasmid S. Enteritidis strains infecting poultry under field conditions.
Émergence de *Salmonella* Enteritidis chez les poulets de chair au Liban : marqueurs épidémiologiques et lutte par exclusion compétitive


Résumé

Les auteurs étudient l'émergence de *Salmonella* Enteritidis dans des élevages de volailles au Liban et identifient des marqueurs épidémiologiques pour quelques-unes des souches isolées. De plus, ils évaluent une méthode d'exclusion compétitive permettant de contrôler les sérotypes de *S. Enteritidis* qui possèdent les marqueurs identifiés dominants.

Dans cette enquête, la procédure de base a consisté à relever les signes et lésions survenant dans onze élevages de poulets de chair et à mettre en culture des prélèvements issus du foie, de la rate et du caecum de dix volailles sélectionnées au hasard dans chaque élevage, afin d'isoler les salmonelles et de déterminer leur type sérologique. En outre, dans quatre élevages de reproducteurs ayant fourni des œufs pour la production de poulets de chair proposés pour l'enquête, des prélèvements de foie, de rate, de caecum et d'oviducte de dix poules ont été mis en culture afin d'isoler les *Salmonella* et de déterminer leur sérotype. Les niveaux de résistance à certains médicaments et le profil plasmidique ont été choisis comme marqueurs épidémiologiques pour *S. Enteritidis*. Une exclusion compétitive a été réalisée en pulvérisant la microflore de barrière sur des poussins d'un jour dans le couvoir, puis en inoculant oralement $2,85 \times 10^5$ unités formant colonies de *S. Enteritidis* par volaille deux jours plus tard. L'exclusion a été évaluée en recherchant la présence de *S. Enteritidis* dans des écouvillonages du cloaque et des prélèvements de rate, de foie et de caecum provenant de chaque animal infecté et traité avec la microflore, ainsi que sur les animaux infectés mais non traités.

Dans les onze élevages de poulets de chair, 112 souches proliférantes de *S. Enteritidis* ont été mises en évidence dans différents organes de poulets qui présentaient des signes et des lésions caractéristiques de salmonellose. Ces souches ont affiché une résistance à la furaltadone et à la gentamycine principalement, un marqueur identifié chez 93 souches (83 %) provenant de neuf des onze élevages considérés. Le même schéma de résistance a été observé dans des souches de *S. Enteritidis* mises en évidence dans un des quatre élevages de poules reproductrices de l'enquête. Le profil plasmidique prévalent dans neuf isolats de *S. Enteritidis*, sélectionnés au hasard dans un groupe de 93 souches (un isolat pour chacun des neuf élevages de poulets de chair) était de 14,1 kilobases (kb) et ~ 50,0 kb, un profil qui rappelle celui observé dans les isolats de *S. Enteritidis* mis en évidence dans les oviductes des poules de l'élevage de reproductrices concerné. L'exclusion a réduit de 3,93 % la mortalité cumulée chez les volailles de 45 jours par rapport à celle du groupe de volailles infectées mais non traitées ($P<0,05$). Chez les volailles de 45 jours, l'exclusion a permis de réduire le taux d'infection par *S. Enteritidis* de 15,6 % dans la rate ou le foie et de 34,4 % dans le caecum ($P<0,05$).

Mots-clés

Liban – Lutte – Marqueurs épidémiologiques – Poulets de chair – *Salmonella Enteritidis*. 
Aparición de brotes de *Salmonella* Enteritidis en pollos asaderos del Líbano: marcadores epidemiológicos y control por exclusión competitiva


**Resumen**
Los autores investigan la aparición de los primeros brotes de *Salmonella* Enteritidis entre pollos del Líbano, determinan los marcadores epidemiológicos de algunas de las cepas Enteritidis aisladas y evalúan un procedimiento de exclusión competitiva para luchar contra la infección de pollos asaderos por microorganismos Enteritidis provistos de los marcadores predominantes identificados.

El procedimiento básico utilizado en la investigación consistió en registrar las lesiones y los signos clínicos presentes en once bandadas de pollos asaderos de otras tantas granjas, y en cultivar después muestras de hígado, bazo e intestino ciego de diez aves por bandada seleccionadas al azar, aplicando a dichas muestras pruebas de aislamiento de *Salmonella* y de tipificación serológica. Se intentó además el cultivo de *Salmonella* y la tipificación serológica a partir de muestras de hígado, bazo, ciego e hisopos de oviducto de diez gallinas (de cuatro bandadas reproductoras de pollos asaderos) que habían puesto los huevos de procedencia de los pollos analizados. Para identificar marcadores epidemiológicos se procedió a determinar los patrones de resistencia a medicamentos y los perfiles plasmídicos de las salmonelas aisladas. La evaluación del método de exclusión competitiva se llevó a cabo rocicando con microflora la incubadora de los polluelos de un día de edad, y procediendo después a la inoculación oral controlada de polluelos de tres días de edad, a razón de $2.85 \times 10^5$ unidades formadoras de colonias de *S. Enteritidis* por individuo. Para evaluar el grado de exclusión se cultivaron hisopos anales y muestras de bazo, hígado e intestino ciego de cada individuo inoculado, comparando a continuación la presencia de *S. Enteritidis* en aves tratadas con microflora y en aves no tratadas.

En once granjas, a partir de los órganos de pollos asaderos que presentaban lesiones y signos típicos de la salmonelosis se obtuvieron 112 cepas invasivas de *S. Enteritidis*. El rasgo de resistencia a medicamentos predominante entre dichas cepas era la resistencia a la furaltadona y la gentamicina, marcador observado en 93 de las cepas (un 83%) y presente en nueve de las once granjas. El mismo patrón de resistencia se observó en las cepas aisladas en las aves reproductoras de una de las cuatro granjas. El perfil plasmídico predominante en nueve microorganismos de *S. Enteritidis*, seleccionados al azar a partir de un conjunto de 93 cepas (un microorganismo por cada una de las nueve granjas de pollos asaderos), era de 14,1 kilobases (kb) y ~50,0 kb, patrón típico y observado en las *S. Enteritidis* presentes en el oviducto de las gallinas de una de las cuatro granjas de reproducción. La exclusión redujo significativamente la mortalidad acumulada en aves de hasta 45 días de edad, en una proporción del 3,93% con respecto a la mortalidad de las aves no tratadas y sometidas a la inoculación de prueba ($P<0,05$). A los 45 días de edad, la exclusión competitiva redujo la tasa porcentual de infección por *S. Enteritidis* de un 15,6% en hígado y bazo, y de un 34,4% en el caso del intestino ciego ($P<0,05$).

**Palabras clave**
Control de enfermedades – Líbano – Marcadores epidemiológicos – Pollos asaderos – *Salmonella Enteritidis*.
References


