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Persistence of *Salmonella enteritidis* in young chickens[1]

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SUMMARY

One- and 7-day-old specific pathogen-free chickens were artificially infected with a field isolate of *Salmonella enteritidis*, phage type 13A. At intervals up to 42 days of age, birds were killed and liver, lung, spleen, brain, yolk sac, testis or ovary, duodenum, jejunum, ileum, caecum and colon collected for culture.

In chickens infected at one day of age and killed 1, 4 and 7 days pi all tissues were infected. Thereafter most non-intestinal tissues (74%) and intestinal tissues (92%) were positive for *S. enteritidis*.

In chickens infected at 7 days of age, all tissues were positive 1 dpi. After post-inoculation day 1, a few non-intestinal tissues (15%) were positive for *S. enteritidis* while 38% of intestinal tissues were positive.

INTRODUCTION

There has been a marked increase in cases of *Salmonella enteritidis* infections in man during the past 20 years (Anusz, 1980; St. Louis *et al.*, 1988). Much of this increase has been associated with eating undercooked or raw eggs. Transovarian transmission is probably the primary means of its spread to man (Hopper & Mawer, 1988; Lister, 1988; St. Louis *et al.*, 1988). *S. enteritidis* infection in adult chickens produces few clinical signs (Hopper & Mawer, 1988), but in young broiler chickens it may cause increased mortality and the culling of large numbers of chickens (O'Brien, 1988).

The purposes of this study were to determine persistence of *S. enteritidis* PT13A (organisms isolated from a poultry house suspected as a source of a *S. enteritidis* outbreak in humans) in young chickens and to determine the effect of the age of the chicken on bacterial persistence.

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MATERIALS AND METHODS

Experimental design

Fifty-three 1-day-old and 28 seven-day-old specific pathogen-free (SPF) White Leghorn chickens were infected as described below with 1×10^7 *S. enteritidis* organisms. Twenty-five 1-day-old and 15 seven-day-old chickens were orally inoculated with sterile saline and used as controls. Four infected and two controls were killed on 1, 4, 7, 14, 21, 28, 35, 42 dpi for 1-day-old infected chicks and 1, 4, 7, 21, 35 for 7-day-old infected chicks. Specimens of liver, lung, spleen, testis, or ovary, yolk sac, brain, duodenum, jejunum, ileum, caecum and colon were collected for culture.

Experimental animals

Thirteen-day-old SPF embryonated eggs were obtained from SPAFAS (Reinhart, PA, 17569). After incubation at one day of age, chicks were moved to cages with wire mesh floors. *Salmonella* culture-negative, unmedicated feed and water were given *ad libitum.*

Bacteria

A strain of *S. enteritidis*, PT13A, isolated from environmental samples taken from a chicken house was obtained from the Maryland Animal Health Diagnostic Laboratory, College Park, Maryland. Bacterial stock cultures were stored on nutrient agar (BBL, Becton Dickinson Company) and prior to experimental use were grown in nutrient broth (BBL, Becton Dickinson Company) at 37°C for 18 h. Cultures were washed in sterile physiological saline for 15 min at $200 \times g$. The supernatant was removed and the pellet resuspended in sterile physiological saline. Using a spectrophotometer (Model 390-Sequoia Turner), the absorbance reading was adjusted to obtain 53×10^7 organisms per/ml. Chicks (53 1-day-old and 28 7-day-old) were orally inoculated, directly into the crop with 0.2 ml (1×10^7 organisms) with controls (25 one-day-old and 15 7-day-old) receiving 0.2 ml sterile physiological saline only. After killing or death, sequentially, selected tissues (see above) were collected aseptically and placed in sterile whirlpool bags. The brain was collected with separate instruments. Instruments used to open the carcass were different from those used to collect tissue samples. Sterile saline was added and tissues were crushed in a stomacher for 15 s. Using a sterile cotton swab, this mixture was swabbed on to Brilliant green agar plates, and Brilliant green agar plates with 1 ug/ml novobiocin (BBL, Becton Dickinson Company). Swabs were placed in selenite broth (BBL, Becton Dickinson Company) for 18 to 24 h at 37°C. When primary cultures were *S. enteritidis* negative the selenite cultures were streaked on the above selective agars. Isolated organisms were confirmed as Salmonellas by use of Gram stain, lysine and triple sugar iron assays, serological group typing and biochemical strip profiles (API Analytab Products, Plainview, New York 11803). Several randomly selected cultures taken from chickens at various ages and the stock culture were identified as *S. enteritidis* by the National Veterinary Services Laboratory, Ames, Iowa.

RESULTS

Mortality

Eleven of 53 chicks (21%) infected at 1 day of age died: two on 2 dpi, three on 3 dpi, two

on 4 dpi, three on 6 dpi and one on 7 dpi. In the 7-day-old group, 2 of 28 (7%) infected chicks died: one died 4 dpi and one on 7 dpi. No control chickens died during this study.

S. enteritidis persistence

All tissues from 1-day-old infected chicks that died during this study were positive for *S. enteritidis*. After 7 dpi, the yolk sac was positive 89% of the time and non-intestinal organs (lung, liver, spleen, reproductive organs and brain) were positive 60 to 80% of the time. All intestinal organs were positive more than 85% of the time, and *S. enteritidis* was always isolated from the caecum and colon. At 42 dpi (market age), *S. enteritidis* was isolated easily from most organs.

All tissues collected 1 dpi from chicks inoculated at 7 days of age were positive for *S. enteritidis*. Thereafter there was a marked decrease in the number of positive non-intestinal organs with the spleen and lungs the only tissues with at least 50% of the samples positive. In contrast, intestinal organs were more frequently positive (Table 1). At 21 dpi, only the liver, ileum, caecum and colon were positive. At 35 dpi (42 days of age—market age), *S. enteritidis* was isolated only from the liver, spleen, yolk sac, caecum and colon.

Table 1. Salmonella enteritidis *culture results from chickens infected at 7 days of age*

dpi[a]	L	LI	S	T-O	Y	B	D	J	I	C	CO
4	2/4	1/4	2/4	0/4	0/4	0/4	3/4	3/4	4/4	4/4	3/4
7	2/4	1/4	2/4	0/4	0/4	0/4	0/4	1/4	2/4	2/4	2/4
21	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	1/4	1/4
35	0/4	1/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4	2/4	1/4
Total	4/16	4/16	5/16	0/16	1/16	0/16	3/16	4/16	7/16	9/16	7/16

[a]dpi=days post-inoculation, L=lung, LI-liver, S=spleen, T-O=testis or ovary, Y=yolk, B=brain, D=duodenum, J=jejunum, I=ileum, C=caecum, CO=colon.

DISCUSSION

This study presents evidence that *S. enteritidis* infections caused by PT13A may be severe and cause high mortality in young chickens. In naturally infected chickens high mortality has been primarily associated with *S. enteritidis* PT4 infection (O'Brien, 1988). While high mortality rates are associated primarily with young chickens (O'Brien, 1988), low mortality rates have been reported in adult layers experimentally infected with *S. enteritidis* PT4 (Timoney *et al.*, 1989).

All tissues cultured from chickens that died of experimental *S. enteritidis* infection were found positive for the bacterium. This is in agreement with Timoney *et al.* (1989). In both studies, chickens died with 7 days of infection. These findings suggest that, in acute infection, culturing tissues from dead birds may be useful in determining if a flock is *S. enteritidis* positive.

S. enteritidis persisted in most organs from 1-day-old infected chickens and fewer organs of 7-day-old infected chicks until 42 days of age (market age). The spleen and liver were the best non-intestinal organs while the caecum and colon were the best overall organs to culture for *S. enteritidis*. The yolk sac was a good organ to culture when chicks were infected at one day of age but not when they were infected at seven days of age. The

widespread invasiveness and persistence of organisms are consistent with previous studies that used various *S. enteritidis* phage types (Turnbull & Richmond, 1978; Williams, 1986; Hinton *et al.*, 1989, 1990).

The results presented here suggest that age at infection plays an important role in the persistence of *S. enteritidis* infection in chickens, that strains other than PT4 may cause severe infections and high mortality in chickens, and that broilers may possibly serve as a source of human *S. enteritidis* infection.

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RESUME

Persistance de *Salmonella enteritidis* chez le jeune poulet

Des poussins exempts d'organismes pathogènes spécifiés, âgés de un à sept jours, ont été artificiellement infectés avec une souche de *Salmonella enteritidis,* phage type 13A isolé sur le terrain. A intervalles réguliers jusqu'à 42 jours, les oiseaux ont été sacrifiés, et le foie, les poumons, la rate, le cerveau, le sac vitellin, les testicules ou les ovaires, le duodénum, le jéjunum, l'iléon, le caecum et le côlon ont été prélevés pour mise en culture.

Chez les poulets infectés à un jour d'âge et sacrifiés à un, quatre et sept jours après inoculation, tous les tissus étaient infectés. La plupart des tissus non intestinaux (74%) et les tissus intestinaux (92%) ont été positifs pour *S. enteritidis.*

Chez les poulets infectés à sept jours d'âge, tous les tissus étaient positifs un jour après inoculation. Par la suite, quelques tissus non intestinaux (15%) ont été positifs pour *S. enteritidis* contre 38% pour les tissus intestinaux.

ZUSAMMENFASSUNG

Persistenz von *Salmonella enteritidis* in Küken

Eintägige und 7 Tage alte spezifisch pathogenfreie Küken wurden mit einem Feldisolat von *Salmonella enteritidis,* Phagentyp 13A, künstlich infiziert. Bis zum Alter von 42 Tagen wurden Küken in Abständen getötet und Leber, Lunge, Milz, Gehirn, Dottersack, Hoden oder Eierstock, Duodenum, Jejunum, Ileum, Caecum und Colon zum Anlegen von Kulturen entnommen.

Bei Küken, die im Alter von einem Tag infiziert und 1, 4 und 7 Tage p.i. getötet worden waren, erwiesen sich alle Gewebe als infiziert. Später waren die meisten Darmgewebe (92%) und die anderen Gewebe (74%) *S. enteritidis*-positiv.

Bei Küken, die im Alter von 7 Tagen infiziert wurden, waren am 1. Tag p.i. alle untersuchten Gewebe

positiv. Nach dem 1. Tag p.i. waren ein paar nicht-intestinale Gewebe (15%), dagegen aber 38% der Darmgewebe *S. enteritidis*-positiv.

RESUMEN

Persistencia de *Salmonella enteritidis* en pollitos

Pollos libres de patógenos específicos de 1 y 7 días de edad fueron infectados artificialmente con una cepa de campo de *Salmonella enteritidis,* fago tipo 13A. Se sacrificaron los pollitos a intervalos y hasta los 42 días de vida, recogiéndose para cultivo hígado, pulmón, bazo, cerebro, saco vitelino, testículos u ovario, duodeno, yeyuno, ileon, ciego y colon.

Todos los tejidos procedentes de los animales infectados a la edad de 1 día y sacrificados a los 1, 4 y 7 días p.i. estaban infectados. La mayoría de los tejidos no intestinales (74%) y de los tejidos intestinales (92%) fueron positivos para *S. enteritidis.*

En los pollos infectados a los 7 días de edad, todos los tejidos fueron positivos 1 día p.i. Tras el primer día p.i. sólo unos pocos tejidos no intestinales (15%) fueron positivos para *S. enteritidis* mientras que lo fueron un 35% de los tejidos intestinales.