ทุนวิจัยงบประมาณแผ่นดินประจำปี 2535

รายงานผลการวิจัย

เรื่อง

อุบัติการของเชื้อ *ซาลโมเนลล่า* ในไก่และผลิตภัณฑ์

โดย

เกรียงศักดิ์ สายธนู

สิงหาคม 2541

ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์
จุฬาลงกรณ์มหาวิทยาลัย

กิตติกรรมประกาศ

โครงการวิจัย เรื่อง "อุบัติการของเชื้อ *ซาลโมเนลล่า* ในไก่และผลิตภัณฑ์" ได้รับงบประมาณสนับสนุนจากเงินทุนวิจัยงบประมาณแผ่นดินประจำปี 2535

ชื่อโครงการ : อุบัติการของเชื้อ *ซาลโมเนลล่า* ในไก่และผลิตภัณฑ์

ชื่อผู้วิจัย : เกรียงศักดิ์ สายธนู

เดือนและปีที่ทำการวิจัย : ตุลาคม 2536

บทคัดย่อ

โรคติดเชื้อ *ซาลโมเนลล่า* ในคนเป็นปัญหาที่สำคัญของทุกประเทศรวมทั้งประเทศไทยด้วย อาหารที่มาจากสัตว์เป็นสาเหตุสำคัญของการเกิดโรค โดยเฉพาะจากไก่และผลิตภัณฑ์ ซึ่งได้มีการศึกษากันมากในต่างประเทศ สำหรับข้อมูล การปนเปื้อนเชื้อ *ซาลโมเนลล่า* จากไก่และผลิตภัณฑ์ในประเทศไทย ยังมีการศึกษากันน้อยมาก ผลศึกษาครั้งนี้ได้แบ่งรายงานออกเป็น 4 ตอน

1. จากการศึกษาเพื่อหา *ซาลโมเนลล่า* จากอาหารไก่จำนวน 812 ตัวอย่าง ซึ่งเก็บจากฟาร์มไก่, ร้านขายอาหารสัตว์ และจากกรมปศุสัตว์ จำนวน 280 , 359 และ 173 ตัวอย่างตามลำดับ พบว่าอาหารไก่ดังกล่าวมีเชื้อ *ซาลโมเนลล่า* อยู่ 8% , 7% , และ 5% ตามลำดับ โดยพบเชื้อ 17 ซีโรวาร์ และ *S. lexinton* , *S. blockley* และ *S. amsterdam* พบมากที่สุด 3 อันดับแรก นอกจากนี้ยังพบเชื้อ ซาลโมเนลล่า ในวัตถุดิบ 16 ชนิด ที่จะนำมาเป็นส่วนผสมของอาหารสัตว์ โดยศึกษาในตัวอย่าง 798 ตัวอย่าง ซึ่งเป็นวัตถุดิบ จำนวน 28 ชนิด พบเชื้อ 28 ซีโรวาร์ และ *S. amsterdamas* จะปนเปื้อนในวัตถุดิบต่างๆ มากที่สุด

2. สำรวจอุบัติการของเชื้อ ซาลโมเนลล่า ในฟาร์มไก่เนื้อ (13 เล้า) ไก่ไข่ (15 เล้า) และฟาร์มพ่อ-แม่พันธุ์ (7 เล้า) ระหว่างปี 2534 และ 2535 โดยการตรวจหาเชื้อจากอาหารไก่ , น้ำ , สวีปจากก้นไก่ ขี้ไก่ และสิ่งรองพื้นคอก พบว่าไก่เนื้อและพ่อ-แม่พันธุ์ทุกเล้ารวมทั้ง 87% ของไก่ไข่มีการปนเปื้อน โดยในเล้าไก่เนื้อจะพบเชื้อ *ซาลโมเนลล่า* ในสิ่งรองพื้นมากที่สุด ในขณะที่อาหารในเล้าไก่ไข่และน้ำกินของไก่ในเล้าพ่อ-แม่พันธุ์ จะมีการปนเปื้อนมากที่สุดในเล้าไก่ดังกล่าว จากจำนวนตัวอย่างที่นำมาแยกหา*ซาลโมเนลล่า* ทั้งหมด 1,488 ตัวอย่าง พบ*ซาลโมเนลล่า*ในสิ่งรองพื้นมากที่สุด 42% รองลงไปคือ น้ำในเล้าไก่ (36%) , อาหารที่เหลือในถาดให้อาหารในเล้าไก่ (28%) น้ำในถังเก็บรวมของฟาร์ม 17% สวีปจากก้นไก่ 13% และจากอาหารไก่ในโกดัง 8% โดยพบ S. blockley S. weltevreden และ S. amsterdam มากที่สุด 3 อันดับแรกตามลำดับ

3. ตรวจหา *ซาลโมเนลล่า* ในเนื้อไก่ดิบ เครื่องใน (หัวใจตับ และกึ๋น) รวมทั้งลูกชิ้นไก่ และไส้กรอกไก่ โดยเก็บตัวอย่างในกทม รวมทั้งหมด 1,135 ตัวอย่าง ที่เก็บจากตลาดสด 9 แห่ง ซูเปอร์มาร์เกต 9 แห่ง และจากโรงงานฆ่าไก่ 4 แห่ง พบ *ซาลโมเนลล่า*ในเนื้อไก่ 66% ในเครื่องใน 86% และในลูกชิ้นรวมทั้งไส้กรอก 10% โดยพบเชื้อทั้งหมด 46 ซีโรวาร์ และอีก 1 สายพันธุ์ที่โคโลนีขรุขระ ซีโรวาร์ที่พบบ่อย 5 อันดับแรกในเนื้อไก่ คือ *S.blockely*, *S.virchow* , *S. enteritidis* , *S.hadar* และ *S.paratyphi B* จำนวน 14, 12, 12, 9 และ 9% ตามลำดับ ซีโรวาร์ที่พบบ่อยในตับ หัวใจ และกึ๋น คือ *S.virchow*, *S. kentucky*, *S. entiritidis*, *S. agona* และ *S.blockley* จำนวน 15, 13, 12, 12 และ 11% ตามลำดับ สำหรับในไส้กรอกและลูกชิ้นไก่พบ S.derby มากที่สุดคือ 33%

4. จากไข่ไก่จำนวน 830 ตัวอย่าง (1 ตัวอย่างประกอบด้วยไข่ไก่ 3 ฟอง) พบว่าไข่ไก่มีการปนเปื้อนเชื้อ *ซาลโมเนลล่า*ที่เปลือก 13.2% ในเนื้อไข่ 3.9% และทั้งที่เปลือกและน้ำไข่ 0.4% โดยพบว่าไข่ไก่จากฟาร์มไก่ไข่ 86 ตัวอย่าง จะมีการปนเปื้อน ซาลโมเนลล่า ที่เปลือกและในเนื้อไข่จำนวน 3.5% และ 1.2 % ตามลำดับ โดยพบเชื้อ 24 ซีโรวาร์ และพบ S. *Salmonella cerro, S. amsterdam* และ *S. typhimurium* มากที่สุด 3 อันดับแรก คือ 4.8% 4.3% และ 1.4% ตามลำดับ และพบ S.enteritidis ในไข่ 2 ตัวอย่างเท่านั้น

Project title : Prevalence of *Salmonella* in Chicken and Their Products

Name of Investigator : Kriengsag Saitanu

Year : October 1993

Abstract

Non-typhoidal Salmonellosis in human is one of the important food borne disease world wide including Thailand . Food from animals were the main source of human infections. Serveral studies on the occurence of salmonellae were reported. However, a few studies have been done in Thailand. The purposes of this report were to elucidate the prevalence of Salmonellae in poultry and their products.

1. A total of 812 poultry feed were examined for Salmonellae. The samples were from poultry farms (280), retail feed shops (359) and Department of LivestockDevelopment (DLD) (173). It was found that 8%, 7% and 5% of the samples from the above sources were positive for Salmonella. Seventeen serovars were confirmed of which *Salmonella* lexington, *S. blockley*, and *S. amsterdam* were the predominant organisms. In addition to the poultry feed, 798 samples of 28 different raw materials used in preparation of animal feed were also tested. Sixteen items of the ingredients were positive. Of the 28 serovars identified, *S. amsterdam* was the most common serovar.

2. Salmonellae were detected in thirteen broiler flocks, 15 layer flocks and 7 parent breeder flocks in Thailand from October 1991 to August 1992. Salmonellae were isolated from samples of feed, drinking water, cloacal swabs, faeces and litter from all broiler and breeder flocks, and 87% of the layer flocks. From broiler flocks, litter samples were more frequently contaminated than other samples, while feed left over in the layer house and drinking water in the parent breeder house were the most commonly contaminated. Of the total of 1,488 samples examined from all flocks, salmonellae were recovered from samples of litter (42%), water in drinking troughs (36%), feed left over in the feed trays (28%) , water in the main tanks (17%) , cloacal swabs (13%) and stock feed(8%). The most common serovars associated with

the broiler, layer and parent breeder flocks were *Salmonella blockley*, *S. weltevreden* and *S. amsterdam* respectively.

3. A study was conducted to determine the presence of salmonellae in raw chicken meat, giblets (liver, heart, gizzard) and cooked chicken products (meatballs and sausages) in Bangkok. A total of 1,135 samples, collected from nine open markets, nine supermarkets and four poultry processing plants, were examined.

Salmonellae were isolated from 467 (66%) of 705 chicken meat samples, 190 (86%) of 221 of giblets and 21 (10%) of 209 cooked products. Out of 678 tested isolates, 46 serotypes and one rough strain were found. The five most common serotypes isolated from chicken meat were *Salmonella blockley, S.virchow , S.enteritidis, S.hadar* and *S.paratyphi* B ; which accounted for 14, 12, 12, 9 and 9%, respectively. The major isolates from giblets were *S. virchow, S. kentucky, S. enteritidis, S.agona* and *S. blockley* (15, 13, 12, 12 and 11%). *Salmonella derby* (33%) was the serotype most often isolated from the cooked poultry products.

4. Two thousand four hundred and ninety eggs wre collected from retail markets in 6 provinces and from laying hen farms in 3 provinces. Eggs were pooled in groups of 3 to obtain 830 samples for testing. Isolation of salmonellae was made from both egg shell and egg contents. Eggs from retail markets were contaminated with salmonellae on egg shell. (13.2%) and in egg contents (3.9%). Three (0.4%) samples yield positive result both on egg shells and egg contents. Of the 86 samples from laying hen farms, salmonellae were found on egg shells and in egg contents, 3.5% and 1.2% respectively. From the 134 strains tested, twenty-four serotypes were confirmed. *Salmonella cerro. S. amsterdam* and *S. typhimurium* were predominantly encountered, 4.8%, 4.3%, and 1.4% respectively. Only two samples were contaminated with *S. enteritidis.*

สารบัญ

คำนำ

มีรายงานจำนวนมากที่ได้ศึกษาถึงสาเหตุของ Non-typhoidal Salmonellosis ในผู้ป่วย และพบว่าไก่และผลิตภัณฑ์ โดยเฉพาะไข่เป็นสาเหตุของโรคที่พบได้บ่อย ซึ่งรายงานดังกล่าวเป็นผลการศึกษาในต่างประเทศ ในประเทศไทยได้มีการศึกษาถึงการระบาดของเชื้อซาลโมเนลล่าหลายครั้ง อาหารเกือบทุกชนิดพบว่ามีการปนเปื้อนเชื้อนี้ นอกจากนี้ยังมีรายงานว่าพบเชื้อนี้ในกิ้งก่าและอีกัวนา

สำหรับการปนเปื้อนของเชื้อ *ซาลโมเนลล่า* ในไก่และผลิตภัณฑ์ ยังมีการศึกษากันน้อยมาก โดยเฉพาะการปนเปื้อนเชื้อในระบบการเลี้ยงไก่ ในไข่ และผลิตภัณฑ์ เช่น ลูกชิ้น และไส้กรอก ดังนั้น การวิจัยครั้งนี้ จึงเป็นการศึกษาการปนเปื้อนเชื้อ *ซาลโมเนลล่า* ในระบบการผลิตไก่ โดยการศึกษาจะครอบคลุมตั้งแต่ฟาร์มจนถึงผู้บริโภค โดยมีจุดประสงค์ที่สำคัญดังนี้

1. หาอุบัติการปนเปื้อนของเชื้อ*ซาลโมเนลล่า*ในอาหารไก่และวัตถุดิบ
2. ศึกษารูปแบบการกระจ่ายของเชื้อ *ซาลโมเนลล่า* ในฟาร์มเลี้ยงไก่เนื้อ ไก่ไข่ และพ่อ-แม่พันธุ์
3. ศึกษาหาระดับการปนเปื้อนของเชื้อ *ซาลโมเนลล่า* ในเนื้อไก่ดิบ และผลิตภัณฑ์ที่สุกแล้ว
4. ตรวจหาการปนเปื้อนเชื้อ *ซาลโมเนลล่า* ในไข่ไก่

เพื่อให้ง่ายและสะดวกในการวิเคราะห์ข้อมูล จึงได้รายงานผลการศึกษาออกเป็น 4 ตอน แต่ละตอนได้มีการวิเคราะห์ วิจารณ์ อย่างละเอียด และได้ตีพิมพ์ในวารสารระดับนานาชาติแล้ว ผู้วิจัยจึงนำบทความที่ตีพิมพ์ดังกล่าวมานำเสนอเป็นบทๆดังนี้

บทที่ 1 Isolation of Salmonellae from Poultry Feed and Feed Ingredients in Thailnad, ตีพิมพ์ใน Jurnal Veterinar Malaysia. 1994, 6(1), 21-24.

บบที่ 2 Prevalence of Salmonellae in Broiler, Layer, and Breeder Flocks in Thailand. ตีพิมพ์ใน Tropical Animal Heallh Production. 1996, 28, 174-180.

บทที่ 3 Occurrence of Salmonellae in Raw Broilers and Their Products in Thailand. ตีพิมพ์ใน Journal of Food Protection. 1994, 57(9), 808-810.

บทที่ 4 Detection of Salmonellae in Hen Eggs in Thailand. ตีพิมพ์ใน Southeast Asian Journal Tropical Medicine and Public Health. 1994, 25(4), 324-327.

บทที่ 1

ISOLATION OF SALMONELLAE FROM POULTRY FEED AND FEED INGREDIENTS IN THAILAND

K. Saitanu[1] and J. Jerngklinchan[2]

[1]*Department of Veterinary Public Health and* [2]*Division of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.*

SUMMARY

A total of 812 poultry feed were examined for Salmonellae. The samples were from poultry farms (280), retail feed shops (359) and Department of Livestock Development (DLD) (173). It was found that 8%, 7% and 5% of the samples from the above sources were positive for Salmonella. Seventeen serovars were confirmed of which *Salmonella lexington*, *S. blockley*, and *S. amsterdam* were the predominant organisms. In addition to the poultry feed, 798 samples of 28 different raw materials used in preparation of animal feed were also tested. Sixteen items of the ingredients were positive. Of the 28 serovars identified, *S. amsterdam* was the most common serovar.

Keywords: Fish meal, poultry feed, *Salmonella*

INTRODUCTION

Our previous studies indicated that poultry meat and eggs in Thailand were highly contaminated with *Salmonellae* (Jerngklinchan *et al.*, 1994 ; Saitanu *et al.*, 1994). In order to control Salmonellae in poultry products, the application of HACCP (Hazard Analysis Critical Control Point) was recommended (Simonsen *et al.*, 1987). The organism might exist in one or more of the poultry production chain and poultry feed was considered as one of the major contaminants (WHO, 1986; Simonsen *et al.*, 1987). However, there is a paucity of information in Thailand on the occurrence of *Salmonellae* in raw materials used for poultry feed preparation and finished feed. Therefore, the purpose of this study is to examine the incidence of *Salmonellae* in raw materials collected from feedmills and finished poultry feed.

MATERIALS AND METHODS

Animal feed ingredients

Seven hundred and ninety-eight samples of 28 different raw materials were collected from four feedmills which produced about 50% of the poultry feed in Thailand. Samples were collected immediately after arrival at the feedmill to avoid cross contamination. The samples were collected during March through May 1992. All samples were tested within one week after arrival at the laboratory.

Poultry feed

Eight hundred and twelve samples of finished poultry feed were collected during March 1991 through August 1992. They came from retail feed shops (359 samples), poultry farms (280) and Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives (173). Samples from DLD were collected by feed control officials during routine monitoring for feed quality control. Samples from poultry farms were collected as unopened samples. Samples were tested immediately or otherwise kept 2 weeks after arrival at the laboratory.

Isolation and identification of Salmonellae

Twenty-five to fifty grams of the ingredients and finished poultry feed were weighed into a sterile Stomacher bag. Buffered peptone water was added 9 times (v/w) of the weighed sample and homogenised for 1 min in Stomacher 400 (Seward Medical, London) and then incubated for 18 h at 37°C. With a 1 mL pipette, 0.1 mL of the pre-enrichment broth was inoculated onto modified semi-solid Rappaport-Vassiliadis (De Smedt and Bolderdijk, 1987) but without the incorporation of novobiocin. The inoculated plates were kept in a plastic box and tightly covered to prevent evaporation and incubated at 42°C for 18 h. Three colonies of motile organisms or the confluent growth which extended from the inoculation area were stabbed and streaked on triple sugar iron agar (TSI) and lysine iron agar (LIA). Colonies exhibiting typical reaction on TSI and LIA after incubating at 37°C for 18 h were purified and further characterised

biochemically (Elliot *et al.*, 1988). The cultures were then tested by slide agglutination technique using *Salmonella* polyvalent O antiserum. Isolates of known somatic groupings were sent to the WHO National *Salmonella* and *Shigella* Center, Division of Clinical Pathology, Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand, for complete serotyping.

RESULTS

The incidence of Salmonellae in poultry feed is shown in Tables 1 and 2. Seventeen serovars were confirmed. The contamination rate in the feeds from retail feed shops, DLD and poultry farms were 7%, 5% and 8%, respectively. The most frequent serovars found in the samples collected from retail feed shops, DLD and poultry farms were *S. lexington* (20 isolates), *S. amsterdam* (6), *S. blockley* (3) and *S. senftenberg* (3), respectively. The percentage of animal feed ingredients contaminated is given in Table 3. Salmonellae were found in 16 various types of feed ingredients. Among the 3 kinds of raw materials of animal sources, 54 (15%) samples of fish meal were contaminated while 3 out of 16 samples of poultry by-products and 1 out of 10 sample of bone meal were positive. Twenty eight serovars were isolated of which *S. amsterdam* (28 isolates) was the most common (Table 4).

Table 1. Source and percentage of samples from which Salmonellae was isolated.

Place of sampling	No.of samples	+ sample (%)
Retail feed shop	359	25(7)
Department of Livestock Development	173	9(5)
Poultry farm	280	23(8)
Total	812	57(7)

Table 2. *Salmonella* serovars isolated from poultry feed

Serovar	Retail feed shop	Poultry farm	DLD
S. lexington	20	-	
S. amsterdam	-	-	6
S. senftenberg	2	3	-
S. anatum	1	2	1
S. blockley	-	3	-
S. paratyphi B	-	2	1
S. bredeney	-	2	-
S. emek	-	2	-
S. mbandaka	-	2	
S. weltevreden	-	2	-
Others	2[A]	5[B]	1[C]

DLD = Department of Livestock Development, Ministry of Agriculture and Cooperation;
A = *S. poona*, *S. potsdam* (1 each);
B = *S. cerro*, *S. enteritidis*, *S. orion*, *S. tennessee* and rough strain (1 each);
C = *S. rissen* (1)
- = Not found

Table 3. Animal feed ingredients from which Salmonellae was isolated

Raw material	No. of samples	No. (%) positive
Poultry by-products	16	3(19)
Fish meal	371	54(15)
Bone meal	10	1(10)
Brewer's grains	14	9(64)
Sesame meal	2	1(50)
Ipil-ipil	22	7(32)
Rice bran	41	10(24)
Peanut shell (Local)	13	3(23)
Soybean shell (Import)	19	4(21)
Sunflower grain shell	14	2(14)
Tapioca pellet	15	2(13)
Lime-stone	29	3(10)
Soybean shell (Local)	20	2(10)
Cotton seed powder	13	1 (8)
Soybean powder	16	1 (6)
Rapeseed	20	1 (5)
Miscellaneous*	163	0

*Corn grain (53 samples), Craked rice (23), Crushed wheat (21), Corn gluten (14), Kapok meal (12), Coconut meat (10), Pea nut shell (10), Crushed oyster shell (8), Crushed grape (4), Sorgnum (4), Pea nut (2), Crushed rice with shell (2)

DISCUSSION

Contamination of feed naturally or artificially with Salmonellae cause salmonellosis in animals (Schleifer *et al.*, 1984; WHO, 1985). However, elimination of Salmonellae in feed can be achieved by the application of CCP (critical control points) (Simonsen *et al.*, 1987). Reports on the percentage of poultry feed contaminated with Salmonellae varied. Isa *et al.* (1963) reported 24.4% animal feeds were contaminated with Salmonellae. Others found that 4-19% were contaminated (Barbour *et al.*, 1983; Nabbut, 1978; Mulder and Van der Hulst 1983; Sato *et al.*, 1982; Girao *et al.*, 1985). The number of cases (%) in Thailand was much lower than in Canada and Lebanon (Isa *et al.*, 1963; Nabbut, 1978).

Table 4. *Salmonella* serovars isolated from animal feed ingredients

Serovar	Fish meal	Peanut shell	Rice bran	Ipil-ipil	Poultry by-product	Brewers grains	Others	Total
S. anatum	5	-	1	1	-	2	-	9
S. agona	-	-	1	-	-	1	1[A]	3
S. amsterdam	13	-	3	3	1	-	8[B]	28
S. derby	-	1	2	-	-	-	-	3
S. havana	3	-	-	-	1	-	-	4
S. kentucky	1	-	-	-	-	-	2[C]	3
S. mbandaka	-	-	2	-	-	2	-	4
S. orion	2	-	-	-	-	-	1[D]	3
S. paratyphi B	1	-	-	-	1	-	-	2
S. rissen	5	-	-	-	-	-	1[E]	6
S. senftenberg	5	-	-	2	-	-	-	7
S. tennessee	2	1	-	-	-	-	2[F]	5
S. typhimurium	1	-	-	-	-	-	2[G]	3
Others	15[H]	1[I]	1[J]	1[K]	-	4[L]	-	22

A = Lime - stone (1 sample); B = Imported soybean shell (2), Soybean powder (1), Tapioca pellet (2), Rape-seed (1), Lime-stone (2); C = Sunflower grain shell (1), Sesame meal (1); D = Domestic soybean shell (1); E = Bone meal (1); F = Domestic soybean shell (1), Sesame meal (1); G = Imported soybean shell (2); H = *S. schwarzengrund* (3), *S. bareilly*, *S. montevideo*, *S.worthington* (2 each), *S. cerro*, *S. gaminara*, *S. idikan*, *S. infantis*, *S. weltevreden*, *S. regent* (1 each); I = *S. meleagridis* (1); J = *S. meleagridis* (1); K = *S. stanley* (1); L = *S. falkensee* (2), *S. emek*, *S. london* (1 each)

Of the 28 ingredients of feeds, 16 were contaminated Salmonellae contamination of animals meals, fish meal, poultry by-products and bone meal was relatively low. In Sweden, imported fishmeal was contaminated as high as 21.3% depending on the source of the product (Gunnarsson *et al.*, 1974). Several workers reported that the rate of Salmonellae contamination in fish meal and animal by-products ranged from 13-38.4% (Karlsson and Thal, 1974; Morris *et al.*, 1970; Stott *et al.*, 1975; Sato *et al.*, 1982;). We found that *S. lexington* was the most prevalent serovar in poultry feed while *S. amsterdam* was most prevalent in the raw ingredients. These serovars were also found frequently in poultry farms (Saitanu - Unpublished data), poultry meat and eggs in Thailand (Jengklinchan *et al.*, 1994 ; Saitanu *et al.*, 1994). The present study revealed that contaminated feed is one of the major sources of Salmonella infection in poultry production in Thailand.

ACKNOWLEDGEMENTS

This work was partially supported by the Annual Research Grant, Fiscal Year 1991 to Faculty of Veterinary Science, Chulalongkorn University. The authors thank Dr. Yoanta Preugs, Chairman of the Division of animal feed control, Department of Livestock Development, Ministry of Agriculture and Cooperative for supplying the feed samples and Prof. R.A. Robinson for reading the manuscript. We are grateful to Mrs. Aroon Bangtrakulnonth, WHO National *Salmonella* and *Shigella* Typing Center, Ministry of Public Health, Nonthaburi, for identifying and serotyping the cultures.

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RINGKASAN

PEMENCILAN SALMONELA DARIPADA MAKANAN AYAM-ITIK DAN RAMUAN MAKANAN TERNAKAN DI THAILAND

Sejumlah 812 makanan ayam-itik telah diperiksa untuk Salmonela. Sampel diambil daripada ladang ayam-itik (280), kedai makanan ternakan runcit (359) dan Department of Livestock Development (DLD) (173). Kajian ini telah menunjukkan 2%, 7% dan 5% daripada sampel diperolehi daripada sumber tersebut adalah positif untuk Salmonela. Tujuh belas serovar telah disahkan dan daripadanya Salmonella lexington, S. blockley dan S. amsterdam *merupakan organisma yang paling banyak. Selain daripada makanan ayam-itik, 798 sampel daripada 28 bahan mentah berlainan yang digunakan dalam persediaan makanan ternakan juga diuji. Enam belas perkara dalam ramuan makanan didapati positif. Daripada 28 serovar yang dikenalpasti,* S. amsterdam *merupakan serovar yang paling biasa terdapat.*

Trop. Anim. Hlth Prod. (1996) 28, 174–180

PREVALENCE OF SALMONELLAE IN BROILER, LAYER AND BREEDER FLOCKS IN THAILAND

J. SASIPREEYAJAN[1], J. JERNGKLINCHAN[2], C. KOOWATANANUKUL[3] and K. SAITANU[3,4]

[1] *Department of Veterinary Medicine;* [2] *Division of Veterinary Microbiology;* [3] *Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*

SUMMARY

Salmonellae were detected in thirteen broiler flocks, 15 layer flocks and 7 parent breeder flocks in Thailand from October 1991 to August 1992. Salmonellae were isolated from samples of feed, drinking water, cloacal swabs, faeces and litter from all broiler and breeder flocks, and 87% of the layer flocks. From broiler flocks, litter samples were more frequently contaminated than other samples, while feed left over in the layer house and drinking water in the parent breeder house were the most commonly contaminated. Of the total of 1,488 samples examined from all flocks, salmonellae were recovered from samples of litter (42%), water in drinking troughs (36%), feed left over in the feed trays (28%), water in the main tanks (17%), cloacal swabs (13%), and stock feed (8%). The most common serovars associated with the broiler, layer and parent breeder flocks were Salmonella blockley, S. weltevreden *and* S. amsterdam *respectively.*

INTRODUCTION

Salmonellosis is an important public health problem in many countries (WHO, 1985; D'Aoust, 1989) and poultry meat and eggs are considered major sources of infection (Cowden *et al.*, 1989; Humphrey, 1990). Salmonellae are frequently detected in poultry meat, eggs and in poultry processing plants in Thailand (Daengprom *et al.*, 1993; Jerngklinchan *et al.*, 1994; Saitanu *et al.*, 1994). Live birds can be infected and/or contaminated with salmonellae before slaughter (Gorham *et al.*, 1991; Simonsen *et al.*, 1987). Birds infected with salmonellae which cause disease in man may be difficult to detect because they usually show no clinical signs. Infected layer hens can transmit salmonellae vertically via the ovary or oviduct or by faecal contamination of the eggs and subsequent shell penetration (Cox *et al.*, 1973; Humphrey *et al.*, 1989).

This paper reports a study into the prevalence of salmonellae in selected broiler, layer and parent breeder flocks in Thailand.

MATERIALS AND METHODS

The study was carried out from October 1991 to August 1992.

Parent breeder flocks

Seven parent breeder flocks (PS), 3 of layer type and 4 of broiler type, varying in size from 1,000 to 3,500 birds were chosen at random. The following samples were collected, 2 to 3 samples of feed in the warehouse, 5 to 9 samples of feed left over in the bird house, one sample of the drinking water from the main tank, 2 to 4 samples of water left over in the drinking trough in the bird house, 10 to 20 cloacal swabs, 14

[4] Addressee for correspondence.

samples of litter and 20 to 25 eggs from each flock. Water samples (200 ml) were collected in sterile bottles.

Layer flocks

Fifteen layer flocks of 1,000 to 5,000 layers were selected, with up to 2 hens in each cage (30 × 40 cm). The samples collected were as for the breeder parent flocks except faeces from under the cages were collected instead of litter.

Broiler flocks

Thirteen broiler flocks of 5,000 to 9,000 birds per house were sampled as for the breeder parent flocks except no eggs were collected.

Isolation and identification of salmonellae

Eggs

Pooled samples of 3 eggs were placed into a sterile bag with 100 ml of buffered peptone water (BPW), left at room temperature for 30 min and subsequently gently rubbed through the bag for one to 2 minutes to release bacteria attached to the shell. Eggs were then removed from the bag and placed in 95% ethyl alcohol for 1 min and flamed to disinfect the shell. Eggs were then cracked aseptically and placed into another sterile bag, their contents diluted with 300 ml BPW and homogenized for 1 min in a Stomacher 400® (Seaward Medical, England). The bags containing BPW after washing the egg shells and the emulsion of egg contents were considered as the samples for isolation of salmonellae from either the egg shells or the egg contents, respectively. The samples were incubated for 18 h at 37°C, after which 0·1 ml was inoculated at the edge of the plate onto modified semi-solid Rappaport-Vassiliadis (MSRV) medium (De Smedt and Bolderdijk, 1987) without novobiocin. The inoculated plates were gently tilted to allow the pre-enriched samples to cover the entire circumference of the plates, and incubated for 18 h at 42°C. Three colonies of motile organisms or the confluent growth which extended from the inoculation area were stabbed and streaked on triple sugar iron agar (TSI) and lysine iron agar (LIA). Colonies exhibiting typical reaction on TSI and LIA after incubation at 37°C for 18 h were purified and further characterised biochemically (Elliott *et al.*, 1988). The cultures were then tested by slide agglutination using *Salmonella* polyvalent O antiserum. Isolates of known somatic groupings were sent to the WHO National Salmonella and Shigella Center, Ministry of Public Health, Nonthaburi, Thailand, for complete serotyping.

Water samples

Water samples (200 ml) were centrifuged for 30 min at 3,000 rpm. After pouring off the supernatant, 100 ml of BPW was added to the centrifuge bottle which was shaken to suspend the sediment, transferred to a sterile bottle, incubated for 18 h at 37°C and examined for salmonellae as for egg samples.

Feed and litter samples

Feed and litter samples of about 50 to 100 g were mixed in 9× of BPW in stomacher bags, incubated for 18 h at 37°C and examined for salmonellae as for egg samples.

Cloacal swabs and faeces samples

Ten ml of BPW was added to the tube containing cloacal swabs incubated for 18 h

TABLE I

Prevalence of salmonellae in poultry flocks[1]

Broiler		Layer		Parent breeder	
No. tested	No. +(%)	No. tested	No. +(%)	No. tested	No. +(%)
13	13(100)	15	13(87)	7	7(100)

[1] A flock was considered positive if salmonellae were isolated from any samples.

at 37°C and examined for salmonellae as for egg samples. Faeces samples (25 g) were processed as for feed samples.

RESULTS

The results are summarised in Table I. Salmonellae were detected from at least one sample from all of the broiler and breeder parent flocks and most (87%) of the layer flocks. Of the environmental samples tested, litter from broiler flocks, feed left over in the bird houses from layer flocks and water in the troughs of breeder parent flocks were the most commonly contaminated being 57, 28 and 42%, respectively (Table II). Eggs from layers were contaminated on the shell (4%) and in egg contents (2%). No salmonellae were found in eggs from breeders. Water samples from the main tank from broiler flocks were the most frequently contaminated. The frequency of various salmonellae found in the flocks is shown in Tables III, IV and V. *Salmonella blockley, S. weltevreden and S. amsterdam* were the most common serovars found in broilers, layers and breeder parent stock, respectively.

DISCUSSION

Our studies showed that 100, 87 and 100% of broiler, layer and breeder parent flocks, respectively, were contaminated and or infected with salmonellae. The organisms were frequently found in litter, left over feed and water in the bird houses.

TABLE II

Detection rate of salmonellae from poultry flocks

	Broiler		Layer		Parent breeder			
Samples	No. tested	No. + (%)	No. tested	No. +(%)	No. tested	No. +(%)	Total tested	Total +(%)
Egg shell	–	–	86	3(4)	55	0	141	3(2)
Egg content	–	–	86	1(2)	55	0	141	1(1)
Feed[1]	166	4(2)	39	9(23)	19	5(26)	224	18(8)
Feed[2]	91	30(33)	67	19(28)	52	10(19)	210	59(28)
Water[3]	18	5(28)	19	2(11)	5	0	42	7(17)
Water[4]	44	17(37)	21	5(24)	24	10(42)	89	32(36)
Cloacal swab	211	53(25)	151	3(2)	111	4(4)	473	60(13)
Litter	85	48(57)	27[5]	7(26)	56	16(27)	168	71(42)

–No sample; [1] Collected from unopened sack or in silo.
[2] Collected from the feed tray in the bird house; [3] Collected from the main tank.
[4] Collected from the trough in the bird house; [5] Faeces only.

TABLE III

Salmonellae associated with 13 broiler flocks

Serotypes	Sources						
	A	B	C	D	E	F	Total[1]
S. blockley	2	3	–	9	21	19	54(31)
S. enteritidis	1	2	–	1	9	1	14(8)
S. paratyphi B biovar *Java*	1	4	–	2	5	2	14(8)
S. anatum	–	1	1	–	4	–	13(7·5)
S. amsterdam	–	2	–	–	6	2	10(5·7)
S. senftenberg	–	4	1	1	1	2	9(5·2)
S. havana	–	4	–	–	1	4	9(5·2)
S. emek	–	3	–	1	1	3	8(4·6)
S. montevideo	–	2	–	–	2	3	7(4)
S. poona	–	1	–	1	1	2	5(2·9)
S. thompson	–	1	1	–	–	1	3(1·7)
S. urbana	–	–	1	2	–	–	3(1·7)
S. bredeney	–	–	–	–	1	1	2(1·1)
S. staniey	–	1	–	–	1	–	2(1·1)
S. ohio	–	–	–	–	2	–	2(1·1)
S.I.13.23:z:-	–	–	1	1	–	–	2(1·1)
Rough strain	–	2	–	–	1	–	3(1·7)
Other serovars	–	2[a]	1[b]	2[c]	5[d]	9[e]	16(9·2)
Total[1]	4(2·8)	32(18·4)	6(3·4)	20(11·5)	59(33·9)	53(30·5)	174(100)

[1] No. of isolates (%); A = Feed in silo or in unopened feed sack
B = Feed left over in the bird house; C = Water in the main tank
D = Drinking water left over in the bird house; E = Cloacal swabs; F = Faeces
[a] *S. albany* and S.I.13.23:-:-(1 strain each). [b] = *S. agona* (1)
[d] *S. ohio* (2), *S. singapore*, *S. lexington* and *S. weltevreden* (1 strain each)
[e] *S. krefeld*, *S. virchow*, *S. orion*, *S. london*, *S. westhampton* and S.I.9.12:-:1.5 (1 strain each)

TABLE IV

Salmonellae associated with 15 layer flocks

Serotypes	A	B	C	D	E	F	G	H	Total[1]
S. weltevreden	1	2	1	3	–	–	–	–	7(12·7)
S. tennessee	1	2	1	1	–	1	–	–	6(10·9)
S. mbandaka	3	2	–	–	–	–	–	–	5(9)
S. orion	–	1	–	–	–	3	–	–	4(7·3)
S. blockley	–	2	–	–	–	–	1	–	3(5·4)
S. heidelberg	–	1	–	1	–	1	–	–	3(5·4)
S. isangi	1	–	–	2	–	–	–	–	3(5·4)
S. london	–	–	–	–	–	1	–	1	2(3·6)
S. kentuckey	–	–	–	–	1	–	–	1	2(3·6)
Other serovars	3[a]	11[b]	–	–	3[c]	1[d]	2[e]	–	20(36·4)
Total[1]	9(16·4)	21(38·2)	2(3·6)	7(12·7)	4(7·3)	7(12·7)	3(5·4)	2(3·6)	55(100)

[1] No. of isolates (%).
A–E as legend to Table III, F = Faeces, G = Egg shell, H = Egg content.
[a] *S. standley* (3 strains).
[b] *S. bredeney*, *S. emek*, *S. havana*, *S. paratyphi B* biovar *Java* (2 strains each) and *S. amsteram*, *S. cerro* and *S. urbana* (1 each)
[c] *S. bere* (2) and S.I. 4.12:-:-(1); [d] *S. augustenborg* (1); [e] *S. enteritidis* and *S. thompson* (1 each)

TABLE V

Salmonellae associated with seven parent breeder flocks

Serotype	A	B	C	D	E	Total[1]
S. amsterdam	3	5	–	–	–	8(16·7)
S. virchow	–	1	–	2	5	8(16·7)
S. emek	–	1	5	–	–	6(12·5)
S. hadar	–	1	–	–	3	4(8·3)
S. london	–	–	–	1	3	4(8·3)
S. stanley	–	–	–	–	4	4(8·3)
S.I.1,4,5,12:i:-	–	–	–	–	3	3(6·3)
S.I.4,5,12:i:-	–	–	3	–	–	3(6·3)
S. anatum	1	–	1	–	–	2(4·2)
S. isangi	–	–	2	–	–	2(4·2)
S. blockley	–	–	–	–	1	1(2·1)
S. eastbourne	–	1	–	–	–	1(2·1)
S. kentuckey	–	–	–	1	–	1(2·1)
S. weltevreden	1	–	–	–	–	1(2·1)
Total[1]	5(10·4)	9(18·8)	11(22·9)	4(8·3)	19(39·6)	48(100)

[1] No. of isolates (%)
A = Feed in unopen feed sack; B = Feed left over in the bird house
C = Drinking water left over in the bird house; D = Cloacal swab; E = Litter
– = Not found

The high percentages of 37, 24 and 42% of *Salmonella* positive samples of water from the trough in the broiler, layer and breeder parent houses, respectively, compared with the lower rate of *Salmonella* positive samples of water in the main supply tanks indicated a build up contamination. The high contamination rates of salmonellae in drinking water left over in the chicken houses may have resulted from cross contamination from the chicken faeces and floor litter. Water supplies in the broiler farms was surface water and 28% samples were contaminated with salmonellae, whereas water for layers and breeder flocks was underground water and had much lower rates of contamination, 11 and 0%, respectively. Surface water should therefore be treated before use, e.g. by chlorination which eliminates salmonellae in drinking water (Poppe *et al.*, 1986).

The great variation in contamination rates of feed before administration to the birds and those left over in the bird houses is an interesting point. Broilers were fed commercial pellets while layers and breeders were fed a mixture of various raw materials with premix. Raw materials may be contaminated with various serovars of *Salmonella*: Saitanu and Jerngklinchan (1995) found that 16 items out of 28 raw materials used in preparation of animal feed were contaminated with salmonellae including brewers grains (64%), ipil-ipil (32%), rice bran (24%), pea nut shell (23%), soybean shell (21%), fish meal (15%), poultry by-products (19%), sunflower grain shell (14%) and tapioca pellet (13%). Peilettisation, however, eliminates salmonellae in feeds (WHO 1990). Therefore, the higher rates of *Salmonella* contamination in the feed of layer and breeder flocks is not surprising as it is composed of raw materials and non peileted premix. Despite the low level of contamination in feed being supplied to broilers, it became heavily contaminated in the broiler house, although there was comparatively little change in the levels of contamination in equivalent feeds in the layer and breeder houses. This is understandable in layers as they were kept in cages with no faeces build up. However, there was also no build up in breeders which

were reared on litter like the broilers. These results may be due to the feeding systems; in the breeder houses automatic feeders were used while in the broilers, conventional feeders were used. Normally there would be little or no feed left over in the automatic feeder, but large volumes of feed were left over in the broiler house and the feed trays were not cleaned. During sampling, feed left over in the breeder houses was collected about one hour after feeding but in broiler houses, feed left over was collected at least 4 hours after feeding. This delay in collection would have increased the chance of cross contamination from litter or faeces.

Litter in the broiler houses was more contaminated than in the breeder houses and this may be due to the higher rate of *Salmonella* carriers in broilers, 25%, compared to 4% in the breeders, together with the more intensive rearing of broilers. The high contamination rates of salmonellae in litter, feed and drinking water left over in the chicken houses are similar to other reports (Bhatia *et al.*, 1979; Laheiloc *et al.*, 1986; Renwick *et al.*, 1992). The main difference is that the high litter and cloacal rate of contamination in broilers may have been due to the contaminated surface water supply. We believe that salmonellae found in drinking water and feed left over in the bird houses resulted from cross contamination from the chicken faeces. *Salmoneila* may also contaminate feathers and be localised deep in the skin (Thomas and McMukin, 1980) and *Salmoneila* contamination on feathers and skin can contaminate carcasses during processing (Daengprom *et al.*, 1993).

Salmonellae were detected in eggs from layers only. Eggs and egg shell may be infected from the oviduct and at the cloaca. (Boriand, 1975). It is worth noting that the breeders' eggs were free from salmonellae and therefore the breeders may not be a major source of salmonellae in commercial poultry production.

ACKNOWLEDGEMENTS

This work was supported by a grant from National Research Council, Thailand. We extend our thanks to Mrs Aroon Bangtrakulnonth, WHO National Salmonella and Shigella Typing Center, Ministry of Public Health, Nonthaburi, for typing the strains and Professor R. A. Robinson for reading the manuscript.

Accepted for publication May 1995

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PREVALENCE DES *SALMONELLAE* CHEZ LES TROUPEAUX A VIANDE, D'ELEVAGE ET DE REPRODUCTION EN THAILANDE

Résumé—Des *Salmonellae* furent détectées dans 13 troupeaux à viande, 15 troupeaux d'élevage et 7 troupeaux de reproduction en Thaïlande entre octobre 1991 et août 1992. Des *Salmonellae* furent isolées dans les prélèvements de nourriture et d'eau, les prélèvements cloacaux, les fèces et la litière de tous les troupeaux à viande et de reproduction et chez 87% des troupeaux d'élevage. Chez les troupeaux à viande les échantillons de litière furent plus fréquemment contaminé que pour les autres échantillons, en revanche c'est la nourriture pour les fermes d'élevage et l'eau pour les fermes de reproduction qui furent le plus contaminées. Sur un total de 1488 échantillons examinés pour l'ensemble des troupeaux, des *Salmonellae* furent trouvées dans 42% des échantillons de litière, 36% pour l'eau des abreuvoirs, 28% pour la nourriture, 17% pour l'eau des réservoirs principaux, 13% pour les prélèvements cloacaux et pour 8% des stocks alimentaires. Les Salmonellae les plus fréquemment associées avec les troupeaux à viande, d'élevage et de reproduction furent respectivement *Saimoneila blockley*, *S. weltevreden* et *S. amsterdam*.

PREVALENCIA DE SALMONELOSIS EN POLLOS DE CARNE, GALLINAS DE PUESTA Y REPRODUCTORAS EN TAILANDIA

Resumen—Se detectaron salmonelas en 13 granjas de pollos de carne, 15 granjas de ponedoras y 7 granjas de reproductoras en Tailandia en el periodo comprendido entre octubre de 1991 y agosto de 1992. Las salmonelas se aislaron en muestras de comida, agua de bebida, frotis cloacales, heces y yacija en todas las granjas de pollos y reproductoras, y en el 87% de las granjas de ponedoras. La muestra contaminada más frecuentemente en las granjas de pollos fue la yacija, mientras que la comida dejada en el comedero (es decir, no consumida por los animales) y el agua de bebida fueron las más frecuentemente contaminadas en las granjas de ponedoras y de reproductoras respectivamente. De un total de 1488 muestras examinadas, se aislaron salmonelas en el 42% de las muestras de yacija, 36% de las muestras de agua procedente de los bebederos, 28% de las muestras de comida dejada en el comedero, 17% de las muestras de agua procedente del depósito principal, 13% de los frotis cloacales y 8% de las muestras de comida del almacén. Las variedades más frecuentes en pollos, ponedoras y reproductoras fueron respectivamente *Saimonellas blockey*, *S. weltevreden* y *S. amsterdam*.

Journal of Food Protection, Vol. 57, No. 9, Pages 808-810

บทที่ 3

A Research Note

Occurrence of Salmonellae in Raw Broilers and Their Products in Thailand

JAOWAPA JERNGKLINCHAN[1], CHAILAI KOOWATANANUKUL[2], KRIENGSAG DAENGPROM[3] and KRIENGSAG SAITANU[2]*

[1]*Division of Microbiology, Chulalongkorn University;*
[2]*Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University; and*
[3]*Division of Veterinary Public Health, Department of Livestock Development, Ministry of Agriculture and Cooperation, Bangkok, Thailand*

(Received July 23, 1993/Accepted February 18, 1994)

ABSTRACT

A study was conducted to determine the presence of salmonellae in raw chicken meat, giblets (liver, heart, gizzard) and cooked chicken products (meatballs and sausages) in Bangkok. A total of 1,135 samples, collected from nine open markets, nine supermarkets and four poultry processing plants, were examined.

Salmonellae were isolated from 467 (66%) of 705 chicken meat samples, 190 (86%) of 221 samples of giblets and 21 (10%) of 209 cooked products. Out of 678 tested isolates, 46 serotypes and one rough strain were found. The five most common serotypes isolated from chicken meat were *Saimonella blockley, Saimonella virchow, Saimonella enteritidis, Saimonella hadar* and *Salmonella paratypni* B: these accounted for 14, 12, 12, 9 and 9%, respectively, of the strains isolated in this study. The major isolates from giblets were *S. virchow, Salmonella kentucky, S. enteritidis, Salmonella agona* and *S. blockley* (15, 13, 12, 12 and 11%, respectively). *Salmonella derby* (33%) was the serotype most often isolated from the cooked poultry products.

Key Words: Chicken meat, chicken meatball, chicken sausage, salmonellae

Salmonellosis is the most prevalent foodborne disease in many countries worldwide (reviewed by 2). Foods of animal origin, especially poultry and poultry products, have been incriminated in the outbreaks of human salmonellosis (*13*). The rate of salmonellae contamination of broiler carcasses, either from processing plants or retail markets, has been reported to vary from 5 to 100% (*2,9,10*). The isolation of these organisms from carcasses in retail markets in Thailand has been the subject of isolated reports. Rasrinaul et al. detected salmonellae only 10 samples from 130 chicken carcasses (*8*). Later, Kanarat et al. (*6*) found that 27% of 4,990 samples of frozen chicken meat from modern poultry processing plants were contaminated with salmonellae. From these two studies, the prevalence of salmonellae in broiler meat in Thailand was considered to be relatively low.

In this paper, we report on the prevalence of *Saimonella* in chicken meat, giblets and cooked products (meatballs and sausages) collected from retail markets and poultry processing plants.

MATERIALS AND METHODS

Samples.

A total of 705 samples of chicken meat and 221 of giblets (liver, heart and gizzard) were collected during October 1991 through August 1992. Samples were obtained from nine open markets (164 and 116 samples of chicken meat and giblets, respectively), nine supermarkets (188 and 105) and four processing plants (353 samples of raw chicken meat). Cooked products, namely chicken meatballs (84 samples) and chicken sausages (125), also were collected from the supermarkets. The samples were tested upon arrival or were stored at -15°C for no longer than 1 month. Most of the raw chicken meat samples from processing plants were frequently stored at freezer temperature.

Isolation and identification.

Frozen samples were thawed at 4°C overnight. Twenty-five grams of liver, heart, gizzard, skin and muscle of raw chicken meat were weighed and put into a Stomacher bag containing 225 ml of buffered peptone water. After stomaching in a stomacher 400 (Seaward Medical, England), the sample and peptone water were incubated together in the bag for 18 to 20 h at 37°C. After incubation, 0.1 ml of sample was inoculated on modified semi-solid Rappaport-Vassiliadis medium (MSRV) (*3*) (without novobiocin) at the edge of the plate. The inoculated plates were gently tilted to allow the pre-enriched samples to cover the entire circumference of the plates, and then the plates were incubated for 18 to 20 h at 42°C. Three colonies of motile organisms or the confluent growth which extended from the inoculation area from each plate were stabbed and streaked into triple sugar iron agar (TSI) and lysine iron agar (LIA) slants. Colonies exhibited typical reaction on TSI and LIA were purified and further characterized biochemically (*4*). The cultures were then examined using the slide agglutination technique with *Saimonella* polyvalent O antiserum, A-65 (0:2-0:65), A-I (0:2-0:16) and groups B(0:4), C(0:7: 0:8), D(0:9: 0:9,46: 0:9,46,27) and E(0:3,10: 0:13,19). Isolates of

known somatic groupings were sent to the World Health Organization (WHO) National Salmonella and Shigella Center, Division of Clinical Pathology, Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand, for complete serotyping.

RESULTS

Salmonellae were detected in 66% of raw chicken meat, 91% of liver, 80% of heart and 80% of gizzard samples (Table 1). The recovery rates of salmonellae from samples collected from supermarkets was somewhat lower than those from the open-markets. Samples collected from poultry processing plants were less contaminated (51%), than those from the retail markets.

Table 2 shows the levels of salmonellae contamination in chicken meatballs (12%) and sausages (9%)..

Among the 678 *Salmonella* spp. isolated (Table 3), 46 different serotypes were found. The most common serotypes were *S. blockley, S. virchow, S. enteritidis, S. agona, Salmonella anatum, S. hadar, S. kentucky, S. parathyphi* B, *S. derby, Salmonella amsterdam, Salmonella montevideo, Salmonella emek* and *Salmonella stanley.*

DISCUSSION

The 66% detection rate of salmonellae from chicken meat was much higher than previously reported (6,8). As expected, chicken meat and giblets in open markets were more contaminated than those in the supermarkets and from processing plants. In the open markets, chicken meat and giblets are kept separately in large containers under unhy-

TABLE 1. *Salmonellae isolated from chicken meat and giblet*

Sample	Open market	Supermarket	Processing plant	Total samples
	No. of samples Tested positive (%)			
Chicken meat	164 143 (87)	188 144 (77)	353 180 (51)	705 467 (66)
Giblets				
Liver	94 86 (91)	38 33 (87)	NT	132 119 (90)
Heart	8 7 (88)	36 28 (78)	NT	44 35 (80)
Gizzard	14 12 (86)	31 24 (77)	NT	45 36 (80)

NT = not tested.

TABLE 2. Salmonella *from cooked chicken products obtained from supermarkets.*

Samples	No. of samples	Tested positive (%)
Chicken meatball	84	10 (12)
Sausage	125	11 (9)
Total	209	21 (10)

TABLE 3. Salmonella *serotypes from raw and cooked chicken products.*

Salmonellae	Open market		Supermarket			Processing plant	Total		
serotype	CM	G	CM	G	P	CM	CM	G	P
S. blockley	19[a]	15	12	6	0	35	66 (14)[b]	21 (11)	
S. virchow	13	6	31	22	-	13	57 (12)	28 (15)	
S. enteritidis	19	12	23	10	-	14	56 (12)	22 (12)	
S. hadar	3	2	16	9	-	24	43 (9)	11 (6)	-
S. paratyphi B	-	-	4	3	-	36	40 (9)	3 (2)	-
S. agona	25	20	7	2	1	7	39 (8)	22 (12)	1 (5)
S. anatum	21	11	14	9	5	2	37 (8)	20 (11)	5 (24)
S. kentucky	10	15	8	9	1	1	19 (4)	24 (13)	1 (5)
S. emek	1	1	1	-	-	12	14 (3)	1 (1)	-
S. amsterdam	9	1	2	5	-	-	11 (2)	6 (3)	-
S. derby	2	3	8	-	7	-	10 (2)	3 (2)	7 (33)
			1	-	-	9	10 (2)		
S. montevideo	2	6	3	2	-	3	8 (2)	8 (4)	-
Other serotypes	24[a]	13[a]	11[c]	8[b]	7[g]	22[f]	57 (12)	21 (11)	7 (33)
Total	148	105	141	85	21	178	467 (100)	190 (100	21 (100)

CM = Raw chicken meat, G = Giblets, P = Cooked products (meatball and sausage).

[a] = Number of positive sample.

[b] = Number of positive sample (% of positive sample).

[c] = *S. sandiego* (5), *S. senftenberg* (4), *S. poona* (3), *S. muenchen* (2), *S. worthington* (2); *S. albany, S. bredeney, S. hvittingfoss, S. london, S. thomson, S. typhimurium,* I.1,4 : 12:1, and I.6,8 : Z_{10} : - (1 each).

[d] = *S. albany, S. bovismorbificans, S. hvittingfoss, S. infantis, S. london, S. muenchen, S. poona, S. saintpaul, S. sandiego, S. senftenberg, S. worthington, S. typhimurium* and rough strain (1 each).

[e] = *S. ohio* (3), *S. krefeld* (2), *S. falkensee, S. infantis, S. london, S. muenchen, S. panama* and *S. saintpaul* (1 each).

[f] = *S. heidelberg* (2), *S. amsterdam, S. meleagridis, S. panama, S. wandsworth,* I. 9,12 : - and I.6, 8, Z_{10} : - (1 each).

[g] = *S. ohio* (3), *S. krefeld* (2), *S. lexington* and *S. typhimurium* (1 each).

[h] = *S. london* (5), *S. chester* (3), *S. albany* (2), *S. sandiego* (2), *S. amsterdam, S. augustenborg, S. bovismorbificans, S. heidelberg, S. muenchen, S. senftenberg, S. typhimurium,* I.4, 12: -, I.1, 4, 12: -, and I.6, 8 : Z_{10} : - (1 each).

gienic condition. The carcasses are piled up on tables without cooling thereby favoring the growth of endogenous *Salmonella* and other human bacterial pathogens. In the supermarkets, chicken meat was packed in styrofoam, wrapped with transparent plastic and kept in the refrigerators. These conditions not only reduce the potential for cross-contamination but also retard bacterial growth.

The average contamination of raw chicken meat from the four processing plants was 51%. Contamination rate of the samples from processing plants A, B and D were 94, 85 and 75%, respectively, whereas that of samples from plant C was only 16%. It might be explained that the chlorine level at the inlet of chill tanks in plants A, B and D was 50 ppm, while that in plant C was 100 to 200 ppm.

Several investigators reported that chlorination was an effective sanitizer in reducing the number of microorganism, including salmonellae (*1,5,11,12*). The recommended chlorine concentration was 10 to 50 ppm (*14*).

The rate of salmonellae contamination in cooked chicken products was 10% (Table 2). This was higher than the expected. The cooked chicken sausages and meatballs, were kept frozen in the supermarkets. Detection of salmonellae in these products indicated that improper handling either before freezing or during marketing might have resulted in cross-contamination.

Most of the common serotypes, except *S. kentucky, S. amsterdam, S. montevideo, S. emek, S. stanley* and *S. parathyphi* B, found in chicken meat were among the most prevalent 10 serotypes found in human cases of salmonellosis in 1991 and 1992 (Aroon-Personal communication, *7*). *Salmonella enteritidis* first occurred among the ten most common serovars in humans and chickens in 1991 (*7*). The following year, it ranked third among human isolates of salmonellae (Aroon-Personal communication). Contrary to other reports, *Salmonella typhimurium* was seldomly detected in our materials. The present results indicated that poultry products could be considered a major potential source of human salmonellosis in Thailand.

ACKNOWLEDGMENTS

This study was supported by the Annual Research Grant, Fiscal Year 1991 to the Faculty of Veterinary Science, Chulalongkorn University. We thank Mrs. Aroon Bangtrakulnonth, head of WHO National Salmonella and Shigella Center, Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand, for typing the strains and Professor R. A. Robinson for reading the manuscript.

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บทที่ 4

DETECTION OF SALMONELLAE IN HEN EGGS IN THAILAND

Kriengsag Saitanu[1], Chailai Koowatananukul[1], Jaowapa Jerngklinchan[2] and Jiroj Sasipreeyajan[3]

[1]Department of Veterinary Public Health; [2]Division of Veterinary Microbiology; and [3]Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

Abstract. Two thousand four hundred and ninety eggs were collected from retail markets in 6 provinces and from laying hen farms in 3 provinces. Eggs were pooled in groups of 3 to obtain 830 samples for testing. Isolation of salmonellae was made from both egg shell and egg contents. Eggs from retail markets were contaminated with salmonellae on egg shells (13.2%) and in egg contents (3.9%). Three (0.4%) samples yield positive both on egg shells and in egg contents. Of the 86 samples from laying hen farms, salmonellae were found on egg shells and in egg contents, 3.5% and 1.2%, respectively. From the 134 strains tested, twenty-four serotypes were confirmed. *Salmonella cerro*, *S. amsterdam* and *S. typhimurium* were predominantly encountered, 4.8%, 4.3% and 1.4%, respectively. Only two samples were contaminated with *S. enteritidis*, one each from open market and laying hen farm, one on egg shells and the other in egg content respectively.

INTRODUCTION

Human salmonellosis is a public health problem worldwide and food animals including avian species are considered as the major source of these infections. Recently, there have been many reports in criminating hen's eggs as the vehicle of the infection. (Chapman *et al*, 1988; Sharp, 1988; Anon, 1988; 1992; Cowden *et al*, 1989a,b; Perales and Audicana, 1989; Rodrigue *et al*, 1990; Humphrey, 1990). The prevalence of salmonellae in egg products, such as frozen unpasteurized eggs has ranged from 32% (Wilder and MacCready, 1966) to 54% (Garibaldi *et al*, 1969). However, the contamination of whole eggs either in retail markets or on farms was relatively low. Baker *et al* (1980) found that 3 (0.2%) eggs from 1,400 tested samples were positive. Generally, the occurrence of *Salmonella* contamination rarely exceeded 1% (Perales and Audicana, 1989; Humphrey *et al*, 1991). The highest rate of reported occurrence of salmonellae in eggs was in Egypt, where 10% of table eggs were positive (WHO, 1985). In Thailand, no confirmed egg-associated outbreaks of human salmonellosis have been reported although sporadic cases may well escape detection. In order to prevent and control the transmission of this foodborne pathogen via eggs to man, we should know the prevalence of the organism. The purpose of the present work was to assess the incidence of salmonellae in eggs collected from retail markets as well as laying hen farms.

MATERIALS AND METHODS

Tested eggs

Two thousand four hundred and 90 eggs were collected from 14 open markets and 9 supermarkets and 7 laying hen farms from October 1991 through June 1992. Of the 14 open markets, 9 located in Bangkok and one each in Chon Buri, Chachoengsao, Lop Buri, Nakhon Ratchasima and Ang Thong Provinces. All supermarkets were in Bangkok. The seven laying hen farms were in Chon Buri (2 farms), Chachoengsao (2), Nakhon Pathom (1) and Saraburi (2). The numbers of eggs from open markets, supermarkets and farms were 1,701,531 and 258, respectively. The eggs from open markets and farms were frequently visibly contaminated with feces, while those from supermarkets were always clean. The eggs were not refrigerated.

Salmonella isolation and identification

Egg samples for salmonellae isolation were grouped in pools of 3, of a total 2,490 eggs, 830 samples were obtained for analysis. Eggs were placed in sterile bags and then 100 ml of buffered peptone water (BPW) was added. They were left in room temperature for 30 minutes and subsequently gently rubbed through the bag for 1 - 2 minutes, in order to release bacteria attached on the shell. Eggs were then removed from the bag

and placed in 95% ethyl alcohol for 1 minute and flamed to disinfect the shell. Eggs were then cracked aseptically and placed into another sterile bag. The egg contents were diluted with 300 ml BPW and homogenized for 1 minute in a Stomacher 400 (Seward Medical, England). The bags containing BPW after washing the egg shells and the emulsion of egg contents were considered as the samples for isolation of salmonellae from the egg shells and contents, respectively. The samples were incubated for 18 hours at 37°C, after which 0.1 ml was inoculated into modified semsolid Rappaport-Vassiliadis medium (MSRV) (De Smedt and Boiderdijk, 1987). The inoculated MSRV plates were incubated for 18 hours at 42°C and 3 motile colonies were stabbed and streaked on triple sugar iron agar and lysine iron agar. Typical colonies were purified and further confirmation was made as described in the previous report (Jerngklinchan and Saitanu, 1993).

RESULTS

Table 1 shows the prevalence of salmonellae in eggs. Salmonellae were frequently found on the egg shells, from which the recovery rates were 12.2, 16.4 and 3.5% of the samples collected from open markets, supermarkets, and farms, respectively. The egg contents were found to be positive in 4.1%, 3.4% and 1.2% of the samples from open markets, supermarkets and farms, respectively. Only 3(0.5%) samples from open markets showed that salmonellae could be isolated from both egg shells and contents.

Table 2 demonstrates the serotypes of salmonellae. Twenty-four serotypes were confirmed from 134 tested strains. Generally, *S. cerro*, *S. amsterdam* and *S. typhimurium* were commonly found on egg shells and in egg contents no matter what the origins of the samples.

DISCUSSION

It would appear from the present study that the prevalence of salmonellae on egg shells and in egg contents is quite high. The rate of contamination of egg shells from the markets varied from 3.5 - 16.4% while the contamination in egg contents was ranged from 1.2 - 4.1%. The contamination of eggs may occur through transovarian passage. *S.pullorum* and *S. gallinarum* commonly infect the ovaries of laying hens and the organisms can be transmitted in the yolk of the eggs (Bryan, 1968; Pomeroy, 1984; Snoeyenbos, 1984). With other salmonellae there is some controversy as to how often this occurs. Shivapransad *et al* (1990) and Forsythe *et al* (1967) could detect *S. enteritidis* from the shells but not from contents of eggs delivered from experimentally infected hens. However, Cox *et al* (1973), Timoney *et al* (1989) and Humphrey *et al* (1989a) detected salmonellae in the egg contents in their experiments which showed that salmonellae other than *S. pullorum* and *S. gallinarum* could undergo vertical transmission. Infected hens excreted salmonellae in feces and this may contaminate eggs (Forsythe *et al*. 1967; Cox *et al*. 1973). Salmonellae contaminating the shells can multiply and penetrate into the chorioallantoic membranes and yolk sacs (Wil-

Table 1

Prevalence of salmonellae from hen eggs collected from retail markets and layer farms.

Samples location	Open markets (n = 567)	Supermarkets (n = 177)	Laying hen farm (n = 86)	Total (n = 830)
Shell	69(12.2)*	29(16.4)	3(3.5)	101(12.2)
Content	23(4.1)	6(3.4)	1(1.2)	30(3.6)
Shell and content	3(0.5)	0	0	3(0.4)
Total	95(16.8)	35(14.8)	4(4.7)	134(16.1)

Number of positive sample (%)

Table 2

Salmonellae serotypes from hen eggs collected from retail markets and layer farm.

Serotype	Open markets			Supermarket		Farm		Total
	Shell(S)	Content(C)	S and C	S	C	S	C	
S. cerro	26*	6	-	8	-	-	-	40(4.8)**
S. amsterdam	15	12	1	8	-	-	-	36(4.3)
S. typhimurium	2	3	1	-	6	-	-	12(1.4)
S. tennessee	5	1	-	-	-	-	-	6(0.7)
S. mbanbaka	3	-	-	3	-	-	-	6(0.7)
S. singapore	2	-	-	3	-	-	-	5(0.6)
S. emek	3	-	-	1	-	-	-	4(0.5)
S. montevideo	3	-	-	-	-	-	-	3(0.4)
I.6, 7 : l, V :-	-	-	-	-	3	-	-	3(0.4)
I.6, 7 : Z_{10} :-	2	-	-	-	-	-	-	2(0.2)
Other serotypes	4[a]	2[b]	1[c]	2[d]	2[e]	3[f]	1[g]	17(2)
Total	65	24	3	27	11	3	1	134(16.1)

* = Number of samples positive
** = Number of samples positive (percentage)
a = *S. alachua, S. enteritidis, S.infantis* and I.6, 7 : d :- (1 each)
b = *S. alachau* and *S. schwarzengrund* (1 each)
c = *S. potsdaur*
d = *S.abany, S. lexington, S. oslo* and I.6, 7 :- (1 each)
e = *S. agona* and *S. albany* (1 each)
f = *S. blockey, S. enteritidis* and *S. thompson* (1 each)
g = *S. london*

liams and Dillard, 1968; Williams *et al*, 1968; Padron, 1990). Salmonellae remained viable and multiplied at 25°C but not at 4°C or 10°C (Clays and Board, 1991; Lock and Board, 1992). Once the organism survived and grew in the eggs contents, they were able to survive in partially cooked eggs (Humphrey *et al*, 1989b). Eggs in the open markets were unwashed and not refrigerated. These conditions are favorable for the growth of bacteria and penetration into the egg contents. Because of the high rate of contamination, hen eggs may be a vehicle of human salmonellosis in this country. We recommend that eggs should be washed, followed with the application of sanitizing chemicals and kept at refrigeration temperature. 4°C, for prevention the multiplication of salmonellae. Consumers should also be encouraged to cook well.

ACKNOWLEDGEMENTS

This work was supported by the Annual Research Grant, fiscal year 1991. Faculty of Veterinary Science, Chulalongkorn University. We thank Mrs Aroon Bangtrakulnonth, WHO National Salmonella and Shigella Center, Department of Medical Science, Ministry of Public Health, for typing the strains and Prof RA Robinson for reading the manuscript

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สรุป

ผลการศึกษาครั้งนี้สรุปให้ว่า :

1. อาหารไก่สำเร็จรูปมีการปนเปื้อน *ซาลโมเนลล่า* สูงมาก โดยอาหารไก่ที่ฟาร์มเลี้ยงไก่จะพบถึง 8% สูงกว่าตัวอย่างอาหารไก่ที่กรมปศุสัตว์เก็บและที่ร้านขายอาหารสัตว์ ซึ่งพบ 7 และ 5% ตามลำดับ แสดงว่าอาหารไก่ที่ฟาร์มมีการปนเปื้อนเชื้อเพิ่มขึ้น

2. ฟาร์มเลี้ยงไก่เนื้อ พ่อ-แม่พันธุ์ มีเชื้อ*ซาลโมเนลล่า*ทุกฟาร์ม และฟาร์มไก่ไข่ จะมีการปนเปื้อนเชื้อ*ซาลโมเนลล่า* 87% บริเวณที่พบเชื้อนี้มาก คือ สิ่งรองพื้นและน้ำดื่มที่ค้างอยู่ในถาดให้น้ำในเล้าไก่ แสดงว่ามีการแพร่เชื้อนี้ทางน้ำและสิ่งรองพื้น โดยไก่ส่วนใหญ่จะมีโอกาสสัมผัสสิ่งปนเปื้อนทั้ง 2 อย่างดังกล่าวตลอดเวลา

3. เครื่องในไก่มีการปนเปื้อนเชื้อ ซาลโมเนลล่า สูงมากถึง 86% เนื้อไก่ดิบ 66% และผลิตภัณฑ์ที่สุกแล้วก็พบเชื้อนี้ถึง 10%

4. เปลือกไข่ไก่จะปนเปื้อนเชื้อ *ซาลโมเนลล่า*ถึง 13.2% และเนื้อไข่ 3.9%

ผลการวิจัยครั้งนี้แสดงให้เห็นว่าเชื้อ *ซาลโมเนลล่า* ปนเปื้อนในระบบการผลิตไก่ทุกขั้นตอน โดยอัตราการปนเปื้อนจะสูงมาก ดังนั้นหน่วยงานที่รับผิดชอบควรมีมาตรการเร่งด่วนในการควบคุมเพื่อลดการปนเปื้อนเชื้อ *ซาลโมเนลล่า*ในไก่อย่างเร่งด่วน ทั้งนี้เพื่อความปลอดภัยของผู้บริโภคภายในและความเชื่อมั่นของผลิตภัณฑ์จากไก่ ซึ่งจะมีผลโดยตรงต่อการส่งผลิตภัณฑ์ดังกล่าวไปต่างประเทศ