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論　　文　　名	*Salmonella* contamination in layer farm : The consequence of rodent infestation （養鶏場におけるサルモネラ汚染：げっ歯類の侵入が及ぼす影響についての研究）	
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論文要旨

Introduction

Food borne illnesses caused by *Salmonella* is documented to be the leading cause of hospitalization and death in the US (Scallan et al., 2011) and causes half of the food-borne outbreaks from 1992 to 2008 in the UK (Gormley et al., 2010). *Salmonella enterica* serovar Enteritidis (SE) infection in humans is usually associated with the consumption of contaminated eggs (Davies and Breslin, 2004). In most countries, SE is one of the most common serotype isolated from food poisoning cases.

Control measures such as obtaining salmonella-free replacement pullets, in-house cleaning and disinfection and SE vaccination are ensured to address the presence of salmonella in the egg production chain (Toyota-Hanatani et al., 2009; Dewaele et al., 2012). With all the advances and initiatives in *Salmonella* control during the last decade, the bacteria were still being isolated at farm level. Rodents such as roof-rats and mice have been implicated in possible introduction, amplification and spread of *Salmonella* in egg producing farms. In chapter 1, the focus on *Salmonella* monitoring of roof-rats obtained from an infested layer farm. In chapter 2, *Salmonella* monitoring of environmental samples (swabs and air-dusts) and eggs is also conducted to determine the transmission of this bacterium from rodents to layer farm. Lastly, in chapter 3, an *in vivo* experiment is conducted by infecting chickens intratracheally (IT) with *Salmonella* obtained from rodent-infested layer farm. The results could provide better understanding on the transmission and spread of *Salmonella* in layer field and will serve as a useful guide in formulating effective control strategies.

Chapter 1: The isolation of *Salmonella* in roof-rats from infested layer farm

Understanding the longitudinal changes in isolation trends of *Salmonella* from rodents is important for surveillance in intensive layer farming systems. Live caught roof-rats (*Rattus rattus*) were obtained and tested for *Salmonella* from 2014 to 2017. Approximately 1 to 2 grams of cecum was collected aseptically for *Salmonella* analysis using standard procedures. Isolation data from 2014 to 2017 were added to the monitoring results from 2008 to 2013 for overall analysis. Of 1,648 roof-rat samples, 165 (10%; 95% CI 8.1-21.9) were positive for *Salmonella*. Four *Salmonella* serotypes were obtained from roof-rats, in which *S.* Potsdam (62.5%) had the highest isolation rate followed by *S.* Mbandaka (17.6%), *S.* Corvallis (16.4%) and *S.* Infantis (3.7%). Shifting in the predominant serotypes was noted from *S.* Infantis and *S.* Corvallis in 2008 and 2014 respectively, to *S.* Potsdam and *S.* Mbandaka in 2015.

The findings confirmed that *Salmonella* could persist for a long time in poultry environment due to rodent vectors such as roof-rats.

Chapter 2: Relationship between the rodent infestation and isolation of *Salmonella* from layer farm environment and eggs

In previous chapter, it has been confirmed that rodents played a key role in the persistence of *Salmonella* in the poultry environment. To determine the extent of contamination from rodents, layer farm environment samples and eggs were also screened for *Salmonella*. Environmental sampling of layer houses was done by swabbing over the egg belts, dusts, floor litter, and manures. Settle plate method was used to assess the air-dusts for *Salmonella* from all layer houses. Two types of eggs were examined for *Salmonella*: 'nest-run eggs' and 'undergrade' eggs. Nest-run eggs were directly collected from layer houses and were not washed and cleaned. Undergrade eggs were oversized, dirty, or eggs with any visible crack. Confirmed *salmonellae* were further serotyped for agglutination with *Salmonella* O and H antigens.

The isolation results from 2014 to 2017 of this study were added to the previous monitoring results from 2008 to 2013 for analysis. From 2008 to 2017, 512 of 9,990 environmental samples (5.1%; 95% CI 2.2-7.4) were positive for *Salmonella*. Similar to roof-rats, shifting in the predominant serotype pattern was also observed throughout the monitoring period. From 2008 to 2012, *S.* Infantis was the most common serotype followed by *S.* Potsdam, *S.* Mbandaka and *S.* Enteritidis. Then, *S.* Infantis was replaced by *S.* Corvallis and *S.* Potsdam to become the most predominant serotypes in 2013 and 2017 respectively. For air-dust monitoring results, 6,310 DHL agar plates were placed in several locations on each windowless house of which, 56 (0.9%; 95% CI 0.2-1.8) were positive for *Salmonella* from 2012 to 2017. Shifting in predominant serotypes in air dusts was also observed in which *S.* Corvallis was replaced by *S.* Potsdam in 2017. Out of 320,000 nest-run eggs (8,000 batches), two hundred-two (0.06% single egg equivalent; 95% CI 0.02-0.08), and 40 (0.01% single egg equivalent; 95% CI 0.002-0.02) were positive for *Salmonella*. in nest run egg shell and contents respectively. On the other hand, twenty six (0.03% single egg equivalent; 95% CI 0.02-0.05) were positive for *Salmonella* from undergrade eggs. Similar shifting patterns of predominant serotype was also noted from eggs. From 2008 to 2012, *S.* Infantis was observed to be the most common serotype before being replaced by *S.* Potsdam and *S.* Mbandaka in 2013.

The results seemed to indicate that roof rats could played an important role in the persistent *Salmonella* contamination and shift of predominant serotypes in layer farm environment, air, and eggs.

Chapter 3: Intratracheal infection of chickens with *Salmonella* obtained from rodent-infested layer farm

In chapters 1 and 2, the results suggested that the presence of *Salmonella* in air-dusts in rodent-infested layer farm may be an overlooked source of infection in poultry. To verify this hypothesis, we conducted an *in vivo* experiment wherein *Salmonellae* (*S.* Infantis, *S.* Potsdam, and *S.* Mbandaka) obtained from rodent-infested layer farm was compared to known invasive strain *S.* Enteritidis on intratracheally (IT) challenged 3-week-old layer chicks. A total of three hundred sixty one-day-old White Leghorn layer chicks were randomly assigned to treatment groups (*S.* Infantis, *S.* Potsdam, *S.* Mbandaka, and *S.* Enteritidis, respectively), in three independent trials. Chicks were grown up to 21 days (3 weeks) and challenged intratracheally with 10^6 CFU of each assigned *Salmonella* organisms per group (n=30). Chicks (n=5) were humanely sacrificed every 24 h for 6 days post-IT challenge and was cultured for *Salmonella* from lungs, heart, liver, spleen, kidney and cecal contents. In all trials, intratracheal challenge of chicks with *Salmonellae* allowed colonization of lungs and cecal contents at 1 day and 3 day, respectively post-infection in all treatment groups. On the other hand, *S.* Enteritidis was also recovered from heart, liver and spleen of IT-challenged chicks.

The results of the *in vivo* experiment contribute to a better understanding of the importance of the respiratory route in *Salmonella* infection in poultry.

Conclusion:

- Despite implementing control measures including biosecurity and vaccination, rodents played an important role in the transmission of this bacterium to the laying flocks.
- Contamination of air with *Salmonella* in poultry houses represent risk of respiratory infection in chickens as demonstrated by *in vivo* experiment in IT-challenged layer chicks.
- In order to maintain low levels of Salmonella, it is strongly recommended to have a continuous rodent control and monitoring program in poultry to avoid the increases of resident rodent population.

審査結果の要旨

サルモネラによる食中毒は、米国の入院と死亡の主な原因の一つであり、1992 年から 2008 年までの英国での食中毒の半数の症例の原因がサルモネラであることが報告されている。 ヒトにおける *Salmonella* Enteritidis（SE）感染は、卵の汚染が最大の原因と考えられている。近年、養鶏場における環境の清浄化と SE ワクチンの接種により SE による食中毒は減少傾向にある。しかし、過去 10 年間の養鶏場内環境の清浄化にも関わらず、サルモネラは分離され､その原因が養鶏場内のクマネズミ等のげっ歯類によるサルモネラの導入、増殖、および拡散の可能性が報告されている。本研究は、第 1 章にて、過去にサルモネラの汚染実績のある養鶏場から捕獲したクマネズミのサルモネラ保菌状況、第 2 章にて、環境サンプルと卵のサルモネラ汚染状況、および第 3 章にて、クマネズミから分離されたサルモネラの鶏気管内投与による感染実験を実施し、養鶏場でのサルモネラの保菌状況、伝播、および拡散に関する調査を実施し、効果的なサルモネラ制御戦略策定のための基盤情報を収集することを目的とした。

第 1 章では、過去にサルモネラの汚染実績のある養鶏場から捕獲したクマネズミのサルモネラ保菌調査を実施した。調査は、2008 年から 2013 年までの当該養鶏場でのデータ（未発表）と 2014 年から 2017 年に採材したサンプルから得たデータを比較検討した。その結

果、1,648 のクマネズミのサンプルのうち、165（10%）がサルモネラ陽性であった。分離したサルモネラ血清型は、*S.* Potsdam (62.5%) 、*S.* Mbandaka (17.6%)、*S.* Corvallis (16.4%) および *S.* Infantis (3.7%)の 4 種類であった。分離したサルモネラの血清型の汚染状態は、2008 年から 2014 年に *S.* Infantis と *S.* Corvallis の検出率が高く、2015 年に *S.* Potsdam と *S.* Mbandaka が優勢と変化していた。サルモネラは、クマネズミなどの媒介動物により、養鶏場の環境に長期間持続的に存在する可能性があることが示唆された。

第 2 章では、環境サンプルと卵のサルモネラ汚染状況について調査した。調査した 9,990 個の環境サンプルのうち 512 検体（5.1%）がサルモネラ陽性であった。2008 年から 2012 年にかけて、*S.* Infantis が最も高い検出率を示し、*S.* Potsdam、*S.* Mbandaka および *S.* Enteritidis がそれに続いた。その後、*S.* Infantis が *S.* Corvallis と *S.* Potsdam に置き換わり、それぞれ 2013 年と 2017 年に検出率が最も高い血清型となった。塵埃のモニタリング結果については、調査した 6,310 検体のうち 56（0.9%）がサルモネラ陽性であった。塵埃からの検出率が高い血清型は、2017 年に *S.* Corvallis が *S.* Potsdam と変化していた。調査した 32 万個の鶏卵から得た 8,000 サンプル（卵 40 個を 1 サンプル）のうち卵内容物から 200（0.06%）、卵殻から 40（0.01%）のサルモネラが検出された。分離されたサルモネラの血清型は、2008 年から 2012 年にかけて *S.* Infantis が最も検出率が高かったが、2013 年に *S.* Potsdam と *S.* Mbandaka が優勢となった

第 3 章では、第 1 章と第 2 章の結果から、げっ歯類が蔓延する養鶏場では、空気の粉塵中のサルモネラの存在が、感染源である可能性が示唆されたため、クマネズミから分離したサルモネラを鶏の気管内に投与するモデルを用いて感染実験を実施した。実験には、養鶏場から分離した *S.* Infantis、*S.* Potsdam、および *S.* Mbandaka とそれに加え、宿主侵襲性が高い *S.* Enteritidis を用いた。すべての試験群で、感染後 1 日目と 3 日目に肺と盲腸内容物から投与した菌を分離した。 加えて、*S.* Enteritidis は心臓、肝臓、脾臓からも回収された。

本研究において、養鶏場の清浄化や予防接種などの衛生対策を実施しても、げっ歯類を原因とするサルモネラ再汚染が引き起こされる可能性が示唆され、鶏舎内でのサルモネラによる塵埃等による空気汚染は、鶏の呼吸器を介して、卵や環境へのサルモネラ汚染に関与する可能性が示唆された。養鶏場内のげっ歯類は少数のサルモネラを保持している可能性が高く、養鶏場における管理および監視プログラムを実施して、げっ歯類の個体数の増加を避けることが重要であることが改めて確認された。本研究は、獣医細菌学および家禽疾病学の分野に重要な知見を提供するのみならず、本疾病の防疫を通して、養鶏産業へも貢献するものと考えられる。そのため、本論文の審査ならびに最終試験の結果と併せて、博士（獣医学）の学位を授与することを適当と認める。