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論文名	「Rodents:Major risk factor in the transmission and persistence of <i>Salmonella</i> contamination in layer farms(養鶏場のサルモネラ汚染伝搬と維持における主たるリスクファクターとしてのネズミの重要性)」
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## 論文要旨

### Introduction

Microbial pathogens of the genus *Salmonella* are among the leading causes of food-borne illness in the world. Reports of almost 380,000 human and 66,000 nonhuman *Salmonella* isolations worldwide were entered into the WHO global surveillance report database during 2000-2002. In Japan, most cases of bacterial food poisoning cases have generally been attributed to *Salmonella* in the past 20 years. From 2004 to 2006, the top two *Salmonella* serovars associated with human salmonellosis in Japan were *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S. Enteritidis*) and *Salmonella enterica* subsp. *enterica* serovar Infantis (*S. Infantis*).

Most of the outbreaks of *S. Enteritidis* infection are often associated with contaminated eggs and egg products. In addition, *S. Enteritidis* is the only human pathogen that routinely contaminates the egg. *S. Enteritidis* seems to be able to colonize the ovary and oviduct of laying hens and this route of contamination has been identified as major risk factor in the emergence of human illness. Since the primary contamination of *S. Enteritidis* occurs at the farm level, on-farm prevention and control measures and quality assurance programs were initiated in the 1990s.

The concept of Hazard Analysis and Critical Control Point (HACCP) has been generally known to Japanese farmers. The Japanese layer industry has

been carrying out numerous measures to prevent egg contamination in layer houses. These measures include, stocking layer houses with *Salmonella*-negative birds from uninfected breeders, regular monitoring of feed samples and sometimes vaccination against *S. Enteritidis*. However, inclusion of rodents as part of intervention strategies to prevent *Salmonella* contamination has not been fully realized by the Japanese layer industry probably due to limited documentations of significant public health threat that rodents pose in livestock operations. In Japan, roof rats (*Rattus rattus*), are the dominant rodent species in poultry premises. Investigations of the prevalence of *Salmonella* infection among rodents in layer farms are limited and its epidemiological role in the spread of *Salmonella* contamination is not yet fully understood.

In the present study, the prevalence of *Salmonella* spp. in rats found in layer farms was investigated. Additionally, isolation of *Salmonellae* from eggs, environment samples, layer hens, liquid egg samples were also conducted. Pulsed-field gel electrophoresis (PFGE) analyses of *Salmonella* isolates from rats, eggs, environment samples, layer hens, and liquid egg samples were also performed to elucidate the epidemiological role of rats in transmission of *Salmonella* contamination in layer farms. Furthermore, a field experiment was initiated on one of the confirmed *Salmonella*-contaminated farm to determine the effect of rodent control program in reducing *Salmonella* contamination levels in the layer houses.

## **Chapter I. The role of roof rats (*Rattus rattus*) in the spread of *Salmonella* contamination in layer farms**

The prevalence of *Salmonellae* in rodents, eggs, and environment samples of 7 commercial layer farms in eastern Japan was investigated between 2004 and 2006 to determine the role of roof rats (*Rattus rattus*) in the epizootology of *S. Enteritidis* contamination in layer farms. Also, the rodent density of each farm was estimated using visual inspection and Rodent Indexing (RI) developed by Henzler. Several *Salmonella* serovars such as *S. Enteritidis*, *S. Infantis*, *S. Thompson*, *S. Potsdam*, *S. Mabandaka*, and *S. Virchow* were detected from environment samples of different layer farms. *S. Enteritidis* and *S. Infantis* strains were the only serovars isolated from pooled egg samples. Persistent *S. Enteritidis* contamination of environment and pooled egg samples were observed in 3 (Farms A, B, and C) of 7 layer farms. These 3 persistently *S. Enteritidis*-contaminated farms have high rodent populations. A total of 113 (12.6 %) and 158 (17.6%) out of 900 roof rats examined were positive for *S. Enteritidis* and *S. Infantis*, respectively. In addition, 13 (1.4 %) rats from Farm E were infected with *S. Thompson*. There were no rodents caught in Farms D, F, and G.

By PFGE analysis, a variety of Pulsed-field patterns were generated by *S. Enteritidis* isolates from rats, eggs, and environment samples of one contaminated farm (Farm A), however, there were also very closely related *S. Enteritidis* and *S. Infantis* strains observed. In addition, PFGE analysis of *S. Enteritidis* strains from rats, eggs and environment samples of Farms B ( $F$  value=0.92) and C ( $F$  value=1.0) yielded genetically related clones. These results suggest that roof rats are persistent carriers of *S. Enteritidis* and *S. Infantis* and may have an important role in the transmission and persistence of these pathogens inside the layer premises.

## **Chapter II. Comparison of the prevalence of *Salmonella* infection in layer hens among layer farms with low and high rodent density**

There are evidences that *S. Enteritidis* gains access to egg contents by migrating from the cloaca to the reproductive organs, or via trans-ovarian transmission following systemic infection and localization in the ovaries, oviducts or peritoneum of laying hens. Consequently, infected flocks are difficult to identify.

There are limited studies that have documented the prevalence of *Salmonella* in naturally infected layer hens in field conditions. This study was conducted to compare the prevalence of *Salmonella* infection in layer hens housed in layer farms with high rodent populations and with low rodent populations. In addition, PFGE analysis of *Salmonella* isolates from layer hens, rats, eggs and environment samples were conducted to determine the epidemiological link between rats and layer hens in the transmission of *Salmonella* contamination in layer farms. A total of 380 layer hens from 7 commercial layer farms were examined for *Salmonella* infection from 2004 to 2006. Portions of the liver, spleen, kidney, intestine, ovary, oviduct, and cloacal swabs were cultured for *Salmonella* isolation. *S. Enteritidis* strains were only isolated from farms with high rodent density (Farms A, B, and C). *Salmonella* infection rate in layer hens ranged from 5.7 % to 8.8 %. Oviduct (48.0 %) was the organ with the highest isolation rate for *S. Enteritidis* followed by liver. Results of PFGE analysis revealed that *S. Enteritidis* strains from layer chickens had the same Pulsed-field patterns generated by *Salmonella* isolates originating from the resident rat population. These findings suggest that high rodent population is a major risk factor in the transmission and persistence of *S. Enteritidis* infection in layer hens.

## **Chapter III. Epidemiological analysis of *S. Enteritidis* and *S. Infantis* isolates from liquid eggs by Pulsed-field gel electrophoresis**

In order to determine the epidemiology of *Salmonella* contamination in

liquid eggs, *Salmonella* isolates from environment, rodent, layer chicken, and egg samples were compared with isolates from liquid eggs from a liquid egg factory, in which some of the eggs supplied originated from a rodent-infested layer farm. The prevalence of *Salmonella* contamination in unpasteurized liquid eggs was investigated. A total of 1,585 batches of unpasteurized liquid eggs were examined for *Salmonella* contamination. The contamination rates of *S. Enteritidis* and *S. Infantis* strains in liquid egg samples were 1.2 % and 0.2 %, respectively. By PFGE analysis, *S. Enteritidis* and *S. Infantis* isolates from liquid eggs are very closely related to *Salmonella* isolates from rodents, layer chickens, eggs and environment samples of the layer farm. *Salmonella* contamination in liquid eggs can be traced back to the rodent-infested farm.

#### **Chapter IV. Effect of reducing rodent population on *Salmonella* contamination levels in layer houses**

The high rodent population inside the layer farms might be an important factor in the transmission of *Salmonella*. To determine the effect of rodent control program in reducing and ultimately eliminating *Salmonella* contamination in layer houses, a field experiment involving initiation of rodent control measures on confirmed highly rodent-infested *S. Enteritidis*-contaminated layer houses was undertaken for six months. Three groups of 2 layer houses each were examined for *Salmonella* contamination on eggs and environmental samples. Two types of rodent control programs were implemented on groups 1 and 2, while, in group 3 (control group), no rodent control measures were applied. Rodent control measures include repair of rodent-damaged walls and attic of layer houses, application of adhesive traps and rodenticide baiting. Rodent density was also estimated monthly using Rodent Index developed by Henzler. The implementation of rodent control measures resulted in gradual reduction of rodent population inside the layer houses and in turn, *Salmonella* contamination rates of eggs and environmental samples were also significantly reduced.

#### **Conclusions**

1. Roof rats are persistent carriers of *S. Enteritidis* and *S. Infantis* and may have an important role in the transmission and persistence of these pathogens inside the layer premises.
2. High rodent population is a major risk factor in the transmission and persistence of *S. Enteritidis* infection in layer hens.
3. *Salmonella* contamination in liquid eggs can be traced back to the rodent-infested farm.
4. The implementation of rodent control measures resulted in the reduction of

rodent population inside the layer houses and *Salmonella* contamination rates of eggs and environmental samples were also significantly reduced.

## 審査結果の要旨

サルモネラは食材媒介性疾病の主要な病原菌の一つであり、これらの汚染防止は世界的な課題である。日本でも、最近 20 年間の細菌性食中毒事例の多くがサルモネラによると報告されており、中でも重要な血清型は *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S. Enteritidis*) と同 serovar Infantis (*S. Infantis*) であり、ここ数年間に日本で分離された血清型の上位を占めている。多くの *S. Enteritidis* 感染事例が汚染された卵や卵製品と関連し、*S. Enteritidis* の主要な汚染現場は産卵養鶏場であるといわれている。そのため養鶏場での汚染防止対策がとられ、導入ヒナ、餌、鶏舎などの衛生管理をはじめワクチンなどを用いたあらゆる方法が講じられた結果、食中毒の発生率は減少傾向にあるが、未だ、十分とはいえない。

申請者は、これまであまり積極的に捉えられてなかった養鶏場におけるネズミのサルモネラ汚染に関わる役割に着目した。日本ではクマネズミ (*Rattus rattus*) が優勢なげっ歯類であるが、被害の実態についての報告は少ない。そこで、養鶏場におけるネズミとサルモネラ汚染について現場に密着した調査を実施し、さらに、ネズミの被害を抑えることによってサルモネラ汚染を抑制できるかどうかを実際の養鶏場を用いて試験した。成績は以下のように要約される。

第1章では、サルモネラ汚染が確認されていた施設を含む7カ所の採卵養鶏場について、3年に亘って詳細な検査を実施し、以下の結果を得た。各養鶏場では調査期間中に少なくとも1度は鶏群を入れ換えている。7養鶏場のうち3カ所では、卵や環境材料に持続的な *S. Enteritidis* あるいは *S. Infantis* 汚染がみられ、同時にこれらの養鶏場ではヘンツラーの Rodent Index (RI) で比較したネズミの棲息密度が高かった。サルモネラの分離頻度を見ると、環境材料よりもネズミの方が高かった。また、分離菌株のパルスフィールド電気泳動 (PFGE) パターンの比較から、それぞれの養鶏場内での分離株は互いに密接に関連していることが判った。他の4養鶏場では環境材料から *S. Enteritidis* や *S. Infantis* 以外のサルモネラ血清型が分離されたが、卵からは分離されず、ネズミの棲息密度も低かった。これらの結果から、ネズミは *S. Enteritidis* と *S. Infantis* の持続的なキャリアであって、養鶏場内の伝播と病原体の持続的な生息に重要な役割を持つことが示唆された。

第2章では、鶏卵を汚染するサルモネラの血清型が *S. Enteritidis* や *S. Infantis* に集中する機序を解明する一助として、養鶏場の産卵鶏についてサルモネラの臓器分布を調べ、分離株についてネズミ由来株間での PFGE パターンを比較した。7養鶏場のうち汚染のみられた3養鶏場の産卵鶏の臓器から *S. Enteritidis* あるいは *S. Infantis* が検出され、特に卵管からの分離率 (48.0%) が他の臓器よりも高かった。PFGE の結果では、産卵鶏から分離した全ての *S. Enteritidis* 株のパターンはネズミ由来株と一致した。したがって、ネズミに由来する *S. Enteritidis* あるいは *S. Infantis* が産卵鶏を汚染し、卵管に到達して卵を汚染した可能性が示唆された。

第3章では、液卵加工された製品から検出された汚染サルモネラと、養鶏場のネズミが保有

するサルモネラとの関係を調べるために、それぞれの分離株について PFGE パターンとフェージ型を比較した。液卵 1,585 サンプルから *S. Enteritidis* が 1.2%、*S. Infantis* が 0.2%の割合で分離された。これらの菌株と、液卵の由来養鶏場のネズミ分離菌株とを比較したところ、両者は非常に密接に関連することが明らかになり、液卵のサルモネラ汚染は、ネズミに侵害された養鶏場にまで遡ることができることが示唆された。

第4章では、*S. Enteritidis* に汚染された鶏舎を用いてネズミを制御した場合にサルモネラ汚染が軽減されるかどうかを試験した。6鶏舎を3群に分け、金属板でネズミの出入りを遮断し殺鼠剤を用いた群、殺鼠剤のみを用いた群、および無処置群を設けた。6 ヶ月間に亘り、ヘンツラーの RI、および卵と環境材料のサルモネラ分離によって評価した。ネズミの棲息を制御した2つの群では *S. Enteritidis* 汚染の有意な低下がみられ、養鶏場でのネズミの棲息がサルモネラ汚染とその持続に関わっていることが証明された。

以上の結果は、鶏卵のサルモネラ汚染に養鶏場のネズミが深く関与していることを証明したものである。これらの結果は、畜産現場におけるサルモネラ汚染対策上重要な示唆を与えるものであり、獣医衛生学、獣医公衆衛生学に寄与するところが大きい。したがって、本論文の審査および最終試験の結果とあわせて博士(獣医学)の学位を授与することを適当と認める。