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Experimental carrier state of *Salmonella Enteritidis* in B13 chicks. M Duchet-Suchaux, P Lechopier, J Marly, P Bernardet, R Delaunay, P Pardon (INRA, Pathologie Infectieuse et Immunologie, Centre de Tours-Nouzilly, 37380 Monnaie, France)

Carrier state is an association between germ and animal, with or without symptoms. *Salmonella* Enteritidis infection in poultry is an example of carrier state, hard to detect and deleterious for human health. We attempted to describe asymptomatic carriage quantitatively, by experimental oral inoculation of conventional inbred B13 chicks with *S Enteritidis* strain 5556 or *S Enteritidis* strain 1009, which is a mutant strain of strain 5556, resistant to streptomycin and nalidixic acid. Carrier state was quantified by numeration of *Salmonella* in liver, spleen and caeca, once a week for 4 to 12 weeks after challenge, in chicks orally inoculated at 1 d, 1 week or 3 weeks of age. After inoculation of $10^2$ bacteria in 1-day-old chicks, *S Enteritidis* strains 5556 and 1009 reached similar levels in organs and they had similar colonization kinetics. Therefore, *S Enteritidis* strain 1009 was used for subsequent experiments. A maximum of about $10^4$ colony forming units (cfu) per spleen was detected in the 2nd week and less than $7.5 \times 10^3$ cfu per organ in the 5th week. The same kind of kinetics was observed in liver, but level of $10^4$ cfu was reached as soon as the first week. In caeca, *Salmonella* levels persisted at $10^3$ to $10^4$ cfu per organ until at least the 8th week. This carrier state was accompanied with diarrhoea and death; mortality rates were 49 and 36% for *S Enteritidis* strains 5556 and 1009, respectively. Repeating this experiment did not exactly reproduce the same results, with shorter colonization kinetics in organs, lower caeca colonization and with 70% mortality rate. In both experiments the mortality rates were too high. We infected 1-week-old or 3-week-old chicks orally with $10^4$ to $5 \times 10^4$ *Salmonella*. In 3-week-old chicks, *Salmonella* colonization did not exceed $10^5$ cfu per organ. Infection was observed in a few chicks and it lasted less than 2 weeks in spleen and liver and less than 5 weeks in caeca. A $10^8$ dose did not modify these results. Spleen and liver colonization in 1-week-old inoculated chicks was observed in every animal in the 1st week with levels between $10^4$ to $10^5$ cfu per organ. Bacterial levels then decreased to 10 to $10^2$ cfu in the 3rd week in a few animals. At this time, colonization rate was significantly higher in the liver than in the spleen ($P < 0.05$).

In the 4th week, *Salmonella* levels in spleen and liver were less than 10 and 50 cfu, respectively. Enrichments of samples in trypticase soy broth allowed us to detect *Salmonella* in the following organs: liver of 3/10 chicks and spleen of 2/15 chicks in the 4th and 9th weeks, respectively. On the 12th week after inoculation one chick presented *Salmonella* in liver, spleen and ovary with levels of 250, $< 10$ and 20 cfu, respectively. *Salmonella* levels in caeca reached $10^7$ to $10^8$ cfu per organ during the first 4 weeks after challenge and they progressively decreased to $10^3$ to $10^4$ cfu per organ in the 12th week. The caeca colonization rate was 90 to 100% until the 10th week and then significantly decreased. In this experiment the mortality rate was 0%. Those results led us to select the following experimental infection conditions to analyze the mechanisms of latent and persistent infection by *S enteritidis* in poultry: 1-week-old conventional chick orally challenged with $5 \times 10^4$ cfu of *S Enteritidis* strain 1009.

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**Persistent faecal shedding of *Salmonella* bredeney in a dairy farm.**

J Marly, P Pardon, B Marquis (Pathologie Infectieuse et Immunologie, 1)
Salmonella bredeney infection in cows has frequently been reported in France. The carriage of *S. bredeney* was followed after a spontaneous outbreak in an experimental farm comprising 160 milking cows (average). Animals were housed from September, the beginning of the calving period, to April, the beginning of the grazing season. No obvious source of contamination was established. Other animals housed and fed independently remained free of contamination until introduction in the infected herd. During the first 2 months of the outbreak, cases of fever (68% of cows) dysentery (80%) and abortions (6.9%) were associated with *Salmonella* isolations in faeces or in products of abortion. *S. bredeney* was recovered from the milk tank, but individual milk sampling was not practicable. From November 1990 to October 1993, the rectal contents of each animal were analysed bacteriologically at intervals of 1 to 3 months. About 5-g portions of faeces from each animal were enriched in 30 ml selenite broth (18 h, 37°C). Subcultures were plated on *Salmonella-Shigella* agar. From 11 to 13 successive faecal samples taken from 217 milking cows and heifers investigated during the first 13 months of observation, 0–10 (median: 3) samples per animal were positive: 48% (92/193) of the animals were only found twice to be positive. Among 217 animals, 89% excreted *S. bredeney* at least once during the first winter following the outbreak. During the following years, the clinical cases were few or absent and the excretion-rate decreased, being maximal during the calving periods and close to zero during the grazing periods. Most animals did not excrete before the day of calving. Only 5 animals excreted intermittently during at least 2.5 years. Three of these carriers were removed before the 2nd grazing season and slaughtered: *Salmonella* was detected in the 3 animals, especially in digestive tract and in draining lymph nodes, but also in one of these animals in spleen, liver and one retro-mammary lymph node. Heifers housed at distance from adults were probably contaminated by the distribution of wasted feeds of adults. After observation of the first clinical cases, half of the 90 heifers was vaccinated twice with a *S. bredeney* dead vaccine in adjuvant. Local and general reactions followed vaccinations. No difference in the rise and fall of the excretion rate was observed between the vaccinated group and the control group. Calves were removed as soon as possible after birth and received a stored colostrum (first maternal colostrum and then pooled colostrum). No clinical salmonellosis or faecal excretion of *S. bredeney* were observed in calves. Autumn 1993 was the first calving period without faecal or vaginal *Salmonella* excretion detectable with our technique, 3 years after the initial isolation. In several respects, these results are similar to those observed with *S. dublin*, a serotype considered as primarily adapted to cattle.

Experimental intragastric contamination of heifers with *Salmonella typhimurium*. J Marly, P Pardon (Pathologie Infectieuse et Immunologie, INRA Centre de Tours-Nouzilly, 37380 Nouzilly, France)

*Salmonella typhimurium* has been reported from many countries among the most common serotypes isolated from several animal species and man, the incidence remaining fairly constant over the years. This investigation was undertaken to reproduce an asymptomatic faecal excretion of *S. typhimurium* in cattle by a mucosal route of contamination. A spontaneous mutant strain resistant to streptomycin (500 μg/ml) and nalidixic acid (100 μg/ml) was selected to quantify low concentrations of *Salmonella*.