Salmonella, multi-drug resistance and the use of live vaccines

Salmonella is still an important foodborne pathogen and infections in humans are caused by non-host specific or broad-host range salmonella serotypes. These serotypes can asymptomatically colonise the gut of multiple food-producing animal species but may cause intestinal disease in humans.

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Although salmonella infections cause relatively mild but self-limiting symptoms, including diarrhoea, fever and abdominal cramps, large outbreaks can occur and for specific groups (elderly, young children) the infection can be life threatening. Salmonellosis in humans is generally caused by the consumption of contaminated food of animal origin. Poultry products (eggs, meat) are the most important source in addition to porcine meat. Salmonella enteritidis is of particular importance because it can spread to the reproductive tract of layers and contaminate eggs.

A worldwide egg-associated salmonellosis pandemic that started in the 1970s is currently partly under control in many countries, thanks to huge efforts of policy makers and the poultry industry. While Salmonella enteritidis infection levels have decreased in many countries in both humans and chickens (e.g. EU), this serotype is still the major chicken product-derived food poisoning problem worldwide. Besides Salmonella serotype enteritidis, the serotype typhimurium is also causing food-poisoning, due to consumption of contaminated porcine and poultry meat.

In addition to the two predominant serotypes, enteritidis and typhimurium, other serotypes can also cause human gastroenteritis. These are mainly derived from meat sources, and the nature of the serotypes depends on the geographical location, and changes over time.

Rise in salmonellosis

Recently, there has been a rise in human salmonellosis cases caused by serogroup C serotypes, derived from contaminated broiler meat. Indeed, in the EU, Salmonella infantis, a group C serotype, is the most common serotype isolated from broiler flocks and meat, with more than 50% of meat samples being positive for this particular serotype.

The exact origin of these strains is not clear, but both horizontal (environmental contamination, feed) as well as vertical (parent flocks) transmission can likely occur. Specific hallmarks of S. infantis strains (and other serotypes) is the increased antimicrobial resistance. Not only the transmission of antimicrobial resistant bacteria to humans, but also the spread of resistance genes between bacterial species using transmissible DNA vectors is an important issue.

A gene cluster making salmonella strains (typhimurium) resistant to ampicillin, chloramphenicol, sulphonamides, streptomycin and trimethoprim (ACSSuT type) has been known for many years.

Recently, the WHO published a catalogue of 12 bacterial groups for which new antibiotics are urgently needed. One of these is fluoroquinolone resistant salmonella strains.

These antibiotics are used to treat life-threatening salmonella infections in humans and thus resistance is a great concern. The emergence of ESBL-type salmonella strains, resistant to cephalosporins, is another concern. Antimicrobial resistant salmonella are and will further become a significant health risk in certain regions of the world, potentially leading to mortality in humans due to the inability to treat infections effectively.

Serotypes that are highly drug-resistant are, for example, Kentucky, typhimurium and infantis, but not enteritidis. Different papers report Salmonella infantis as a dangerous and multi-drug resistant salmonella serotype and such strains seem to emerge worldwide.

In addition to the general maintenance of good biosecurity management, feed additives have been proposed as interventions that can be used to reduce salmonella colonisation, shedding and ultimately meat contamination. As an example, the use of acidifiers, including butyric acid, has been widely used for this purpose.

Vaccination: the logical tool

To control pathogens, especially in long-living animals such as layers or breeders, vaccination is of course a logical preventive tool.

While vaccines have been commercialised based on enteritidis and typhimurium, group C live vaccines are not yet available. To evaluate whether Salmonella enteritidis and typhimurium-based live vaccines also cross-protect against Salmonella infantis, studies have been carried out with AviPro Salmonella Duo, consisting of enteritidis and typhimurium live vaccine strains.

Three studies were carried out by Eeckhaut et al. at Ghent University. One studied early post hatch protection by administering the dual live vaccine at day of hatch and

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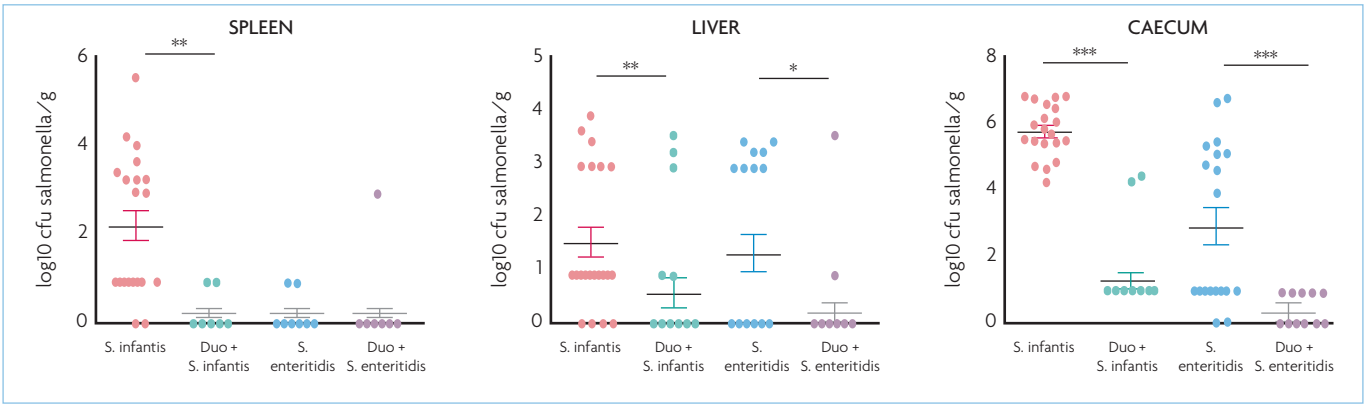
Fig. 1. Caecal and internal organ colonisation by Salmonella infantis (red) and enteritidis (blue) after double dose (day 1, week 6) oral vaccination and challenge at week seven with the respective strains at 10^9 cfu. Sampling was done at week eight.



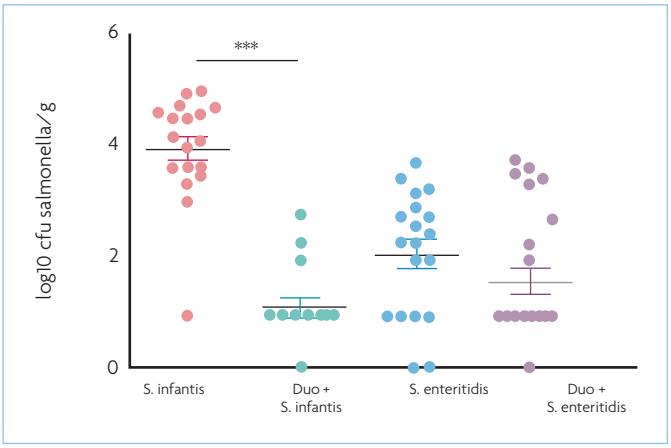


Fig. 2. Caecal colonisation by Salmonella infantis (red) and enteritidis (blue) after triple dose (day 1, week 6 and week 16) oral vaccination and challenge at week 17 with the respective strains at 10^9 cfu. Sampling was done at week 18.

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thus inducing colonisation-inhibition which is described as within-serotype protection. When a live vaccine is orally administered at day of hatch and challenge strains (of the same serotype) are administered shortly thereafter, a form of early colonisation-resistance can be accomplished likely by microbial competition, however exact mechanisms are unclear.

Potentially, it can be of benefit for both broilers and layers. When groups of animals were at day one orally vaccinated with 10^8 AviPro Salmonella Duo, and challenged at day two with 10^5 cfu of either a Salmonella typhimurium, enteritidis or infantis strain, protection against caecal colonisation was mainly seen against the homologous serotypes, and was significant against Salmonella enteritidis.

This is a commonly described phenomenon, and it has been known for a long time that colonisation-inhibition, and thus early protection is an intra-serotype specific event. Protection against spleen colonisation, as marker for systemic spread of salmonella, was seen against all three serotypes.

This has traditionally been explained by attraction of aspecific immune cells to the gut wall, which protect against mucosal invasion by any (serotype-independent) serotype.

Of more importance, in terms of protection against reproductive tract colonisation and subsequently egg contamination, is the use of the existing live vaccines in protecting layers and breeders against colonisation of the gut and internal organs. In two trials, the effect of either a double or triple dose vaccination regime with AviPro Salmonella Duo on gut and internal organ colonisation by Salmonella enteritidis and infantis was evaluated. Double dose vaccination was done orally at day one and week six with AviPro Salmonella Duo, and challenge was performed at week seven with 10^9 cfu of the respective challenge strains.

At week eight (one week post-infection) samples were taken for bacteriological analysis that was performed by plating on selective media and colony counting, and by inoculation on enrichment media so that low levels of bacterial colonisation were also detected.

It was shown (Fig. 1) that AviPro Salmonella Duo significantly decreased colonisation levels of enteritidis and infantis in the caeca and internal organs.

The reduction in caecal colonisation levels was, on average, higher than 3 log units per g tissue, so a more than 10,000-fold decrease in numbers, making these data highly relevant for the field.

Even more important, when the animals received a triple dose regimen (day 1, week 6 and 16) with AviPro Salmonella Duo, were challenged at week 17 with 10^9 cfu of Salmonella enteritidis or infantis, and were sampled at week 18, again significant protection was seen.

Caecal colonisation by Salmonella infantis was significantly reduced in the vaccinated group relative to the control group (Fig. 2).

In summary, AviPro Salmonella Duo thus protects against caecal colonisation by Salmonella infantis.

This kind of cross-protection against non-homologous serotypes is important as this indicates that layer and breeder protection can be rather broad-serotype range instead of serotype-specific, which is important for overall salmonella control. ■

References are available from the author on request

Fig. 3. Caecal colonisation by Salmonella infantis after two doses (challenge at week 7) and triple dose (challenge at week 17).