‘Wetenschappelijke ondersteuning van een prenormatief onderzoek in de voedingssector in het kader van een duurzame ontwikkeling’

STUDIE VAN *SALMONELLA* EN *CAMPYLOBACTER* KRINGLOPEN BIJ DE PRODUCTIE VAN BRAADKUIKENS

Eindverslag

Wetenschappelijke partners:
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Executive summary

Rationale and objectives

The last decade is characterized by a dramatic increase of human cases of salmonellosis and campylobacteriosis in most Western countries. Figures of epidemiological research indicate that about 10 % of human salmonellosis and nearly all campylobacteriosis cases are caused by consumption of contaminated poultry meat. Together with the increase of these two important human food infections, an important persistence of these pathogens is noticed in the production chain of poultry meat. A control program against the presence of *Salmonella* and *Campylobacter* in poultry products has to consider a reduction of the contamination in the whole production chain.

Salmonella and especially *S.* Enteritidis can infect hatching eggs and can so be transmitted from the parent poultry flock to the broiler. This is called vertical transmission. *Salmonella* can also be transmitted horizontally from the environment to the broiler. A policy, focusing only on the vertical transmission by clinical treatment and eradication of the *Salmonella* positive parent flocks, is not effective when the horizontal transmission is not controlled. Literature shows that risk factors for horizontal transmission are fluctuating over time and differ in function of the geographical location of the poultry farms. The research of this project aims to identify the most important *Salmonella* contamination routes in the broiler production chain and to formulate recommendations for an efficient combative program.

The situation differs for *Campylobacter* because this pathogen is only horizontally transmitted to the broilers. Only limited information is available in literature about the *Campylobacter* contamination routes to broilers and poultry meat products and this project aims to add to this knowledge.

Antibiotic resistance patterns often arise in human pathogens and constitute a considerable danger for public health. These resistance patterns are not well known for *Salmonella* and *Campylobacter* isolated from the production chain of poultry meat in Belgium.

Task description

To achieve the objectives of the project a multidisciplinary partnership has been established with the University of Ghent, Veterinarian faculty, Prof. Dr. L. De Zutter (RUG), the Agricultural research centre, Department of animal product quality, Dr. L. Herman and Dr. M. Heyndrickx (DVK-CLO), the Centre for research in veterinary medicine & agrochemistry, Dr. D. Vandekerckhove (CODA) and the UMC Sint-Pieter, Prof. Dr. J.-P. Butzler.

In the period from april 1998 tot march 2000, the *Salmonella* and *Campylobacter* contamination of 18 broiler flocks was followed in detail from the hatchery to the cooled carcasses in the slaughterhouse (see scheme).

Scheme of the production chain. The shaded zones are studied in this project by the indicated partners.

Production chain	Parent flocks	Hatchery	Broiler farm	Slaughterhouse
Sampling time		day 22	6 weeks 3 samplings	Transport Slaughtering process
Sampling of		one-day old chicks, environment	transport of chicks hygiene of rearing house, animals and environment during rearing	Transport containers Carcasses organs
Partners		DVK-CLO		RUG

The *Salmonella* and *Campylobacter* isolates were collected, typed with molecular methods (DVK-CLO and RUG) and from a representative amount of strains, the antibiotic resistance profile was determined (UMC Sint Pieter). The obtained results were analysed by epidemiological statistical methods (CODA).

Results

For the isolation of *Salmonella*, methods with an enrichment in Rappaport Vassiliadis (RV), in 'Diagnostic semi-solid *Salmonella* agar' (Diassalm) and in 'Modified Semi-solid Rappaport-Vassiliadis' media (MSRV) were compared. In total 3150 samples were analysed and a combination of selective enrichment in RV and Diassalm reached a sensitivity of more than 99 %.

Vertical transmission of *Salmonella* was demonstrated in 2 and maybe in 3 flocks. *S.* Enteritidis was isolated from broken egg shells in the hatchery and from the trayliners obtained after transport of one-day old chicks. These strains did not persist in the animals during rearing. *S.* Hadar, isolated from the trayliners did not persist in the broilers. From each of 4 houses a different serotype of *Salmonella* was isolated before arrival of the one-day old chicks: *S.* Virchow, *S.* Blockley, *S.* Hadar and *S.* Infantis. Only the *S.* Hadar and *S.* Blockley contaminations persisted by the animals during rearing. Overshoes were found positive in 10 of the 18 flocks during rearing and obtained a *Salmonella* positive status. The *Salmonella* status was most sensitively determined by the overshoe method. The results indicated, however, that different pairs of overshoes have to be taken to reach a representative sensitivity. In the farm environment, a high contamination level (11 of the 18 farms were positive) was found. In 4 flocks *Salmonella* was isolated from the feed: 2 *S.* Mbandaka, 1 *S.* Blockley and one non-typable isolate. *S.* Blockley persisted in the animals. The most important infection of the broiler flocks happened during the 2 first weeks of rearing, the amount of positive flocks decreased during further rearing. In 12 of the 18 flocks, antibiotics were administered to the broilers during the rearing period. Antibiotic usage influenced the amount of positive overshoes and caecal drops significantly ($p = 0,02$). The intake of movable material in the house after cleaning and disinfection was identified as most important factor for horizontal transmission of *Salmonella* during rearing ($p = 0,08$). The hygiene of the houses, other animals on the farm (inclusive domestic animals, insects, spins, rodents and birds), ditch water, puddles and other surfaces in the environment of the house did not function as a significant source for *Salmonella*. Also the amount of houses on the farm did not influence significantly the *Salmonella* infection of the animals.

With pulsed field gel electrophoresis (PFGE) several *Salmonella* serotypes could be further subdivided in genomic types. *S.* Mbandaka showed the greatest diversity in types; however, only one genomic type was sporadically found in the animals of 2 flocks. In 2 successive flocks in the same broiler house of a rearing farm, the same *S.* Hadar genomic type was dominantly found in the animals; this type can be considered as highly virulent in chickens. This type was already present in the environment before arrival of the one-day-old chicks of the first followed flock. On this rearing farm, another *S.* Hadar genomic type was also present in the environment and in the house before arrival of the one-day-old chicks of the following flock, but this type was not found in the animals and is thus possibly not or less virulent for chickens. The *S.* Enteritidis isolates from the 2 hatcheries and 2 flocks belonged to 2 different genomic types. In one of these flocks, the same *S.* Enteritidis genomic type was isolated from the animals during the whole rearing period. In the other flock, firstly 2 other serotypes were isolated from the animals, and then after 6 weeks rearing a genetically changed *S.* Enteritidis strain (acquisition of a megaplasmid). In a flock followed on a circulation farm, the animals were contaminated with a dominant and a sporadic genomic type of both *S.* Blockley and a non-typeable serotype. The dominant types were transferred to the animals by unsufficient hygiene in the house before arrival of the one-day-old chicks, as well as to other houses by footwear. In 2 other flocks, transfer of the same genomic type of a certain serotype could also be demonstrated, respectively between houses and (probably) via feed to the animals.

At the slaughterhouse level, more *Salmonella* positive samples were isolated. No significant correlation was found between the infection during rearing and the contamination of the slaughtered carcasses. The positive faeces from the transport boxes are mostly derived from the transport boxes themselves. The identity of the slaughterhouse was identified as the most determinative factor for the contamination of the carcasses. Analysis indicated that neither the *Salmonella* status of the flock, nor the evisceration

technique nor the time of slaughtering (slaughtered first or not) had a significant influence. Additional research showed that during slaughtering the feathers were found almost systematically contaminated with *Salmonella* even from flocks with a negative status. In some slaughterhouses the carcasses were already highly contaminated with *Salmonella* after plucking. Further processing resulted in a reduction of this contamination. From molecular typing with PFGE, it could be deduced that in only 5 flocks carcasses were contaminated with the same strain as isolated from the live animals. In one flock, carcasses were contaminated with a *S*. Hadar strain which was before only isolated from faeces of a dog on the rearing farm; possibly, unloading of the flock for slaughter can be responsible for this contamination.

No *Salmonella* isolate showed resistance for cefratoxin, ciprofloxacin and kanamycin. About 30% of the isolates were resistant for streptomycin, ampicillin, amoxillin and tetracyclin, about 12% for nalidixin acid and trimethoprim/sulfamethoxazole. Of the isolates, 42% were resistant for at least 1 antibiotic, 11% for 5 antibiotics. It was striking that all 49 *S*. Hadar strains were resistant for at least 2 antibiotics and most of these were resistant for 3 to 5 antibiotics.

Campylobacter was not found in the hatchery and by the one-day old chicks. Also no isolates were obtained from the rearing house before the arrival of the chicks. The *Campylobacter* status during rearing of the broilers was most sensitively determined by the analysis of caecal drops. The infection of broiler flocks increased continuously during the rearing time. Seven flocks in total were positive for *Campylobacter*; in all cases, *Campylobacter jejuni* was found, in only 1 flock also *Campylobacter coli* was found. In the environment of the house, *Campylobacter* was isolated in 11 flocks. The movable material and especially the footwear of the farmer were determined as significant risk factor ($p = 0{,}036$). The administration of antibiotics reduced the shedding of *Campylobacter* by positive animals. This effect was however not significant as it was for *Salmonella*.

From typing with PFGE and *fla*A-restriction analysis, it followed that each flock was contaminated with its own *C. jejuni* genomic type. *C. jejuni* is thus genetically a very heterogeneous species. The drinking water was frequently contaminated with the same genomic type as found in the animals, which can cause a further spread of the contamination in the flock. In 2 flocks, the animals were contaminated with several *C. jejuni* genomic types; in one of these flocks, 4 types succeeded each other during rearing. Transfer of the same genomic type from the environment or between houses (probably via footwear) could be demonstrated in several flocks.

After slaughtering, 12 flocks were positive for *Campylobacter* in the caecum content and 13 on the carcasses, which indicate an extra contamination during the slaughtering phase. This extra contamination was not correlated with the identity of the slaughterhouse and started by the transport of the animals. The contamination of the carcasses was clearly correlated with the contamination of the animals during rearing and not with the applied evisceration technique and with the time of slaughtering (slaughtered as first flock or not).

None of the 178 tested *Campylobacter* strains were resistant for amoxicillin/clavulaanzuur 2/1 and only 1 strain for gentamycin. For many antibiotics an intermediary resistance was established. About 27% of the strains were resistant for ciprofloxacin, nalidixin acid or tetracyclin, about 8.5% for erythromycin or ampicillin. Only 6% of the strains were resistant for all tested antibiotics, 13 % were resistant for only 1 antibiotic, 27% for 2, 10% for 3, 2 strains for 4 and 1 strain for 5 antibiotics.

Conclusions and recommendations

- For the isolation of *Salmonella* from poultry related samples, a combination of selective enrichment in RV and Diasalm reached a sensitivity of more than 99%.
- Vertical transmission of *Salmonella* still occurs, which indicates the importance of further efforts to control contamination in the parent flocks.

- Our results show clearly that there is a decrease of the relative importance of the first stages in production and an increase of the relative importance of the last stages (transport of broilers and slaughter). The extensive contamination during rearing is easily transferred from the environment to the broilers in the poultry house. It is important to correctly use the hygiene gate and to decontaminate the footwear.
- *Salmonella* contamination in a flock is most sensitively determined by the overshoe method. For this more than 2 pairs of overshoes has to be taken on different sampling times during rearing. Especially the use of antibiotics during rearing decreases the presence of *Salmonella* in the faeces and in the overshoes and has to be considered in control programs.
- The investigation of the presence or absence of *Salmonella* in certain samples and even serotyping are not always sufficient for the exact determination of contamination sources. In many cases, only molecular typing gives the necessary information for epidemiological links. Pulsed field gel electrophoresis with the use of the appropriate enzymes (*Xba*I and *Not*I) is a technique with sufficient resolution for the serotypes encountered in broilers amongst which the clonal serotype *S*. Enteritidis.
- Faeces from transportcontainers cannot be used to detect a *Salmonella* and *Campylobacter* contamination in flocks presented in the slaughterhouse. These faeces samples can be contaminated by unsufficiently cleaned and desinfected containers.
- No correlation exist between the status of the flocks and the contamination of the carcasses in the slaughterhouse. The identity of the slaughterhouse is of significant importance for the carcass contamination with *Salmonella*. An obvious contamination takes place during the first stage of the slaughtering process. The evisceration method and the time of slaughtering (slaughtered as first flock or not) do not have an important influence on the contamination of the final product.
- *Campylobacter* was not isolated from one-day old chicks and in the hatchery. The hygiene of the poultry house did not seem to play an important role in the contamination of the flock. *Campylobacter* is clearly transmitted to the animals during rearing from the environment with the footwear as most important vector. Also the drinking water is an important vector for further spreading of the contamination. This indicates the importance of an efficient hygiene gate and a correct disinfection of the footwear and of the drinking water.
- The presence of *Campylobacter* contamination in a flock is most sensitively investigated by the analysis of caecal drops. The amount of positive drops increases continuously during rearing of a positive flock. As a consequence, the best moment to determine the status of the flock is just before slaughtering. Each positive flock can be contaminated with a different *C. jejuni* genomic type; some flocks can be even contaminated with several (successive) types. The administration of antibiotics during rearing has a reducing effect on the presence of *Campylobacter* in caecal drops. This effect is not significant and more limited than found for *Salmonella.*
- The *Campylobacter* contamination during rearing is quite correlated with the contamination of the final product. This contrasts with the *Salmonella* results. The identity of the slaughterhouse is not a significant factor for the final carcass contamination with *Campylobacter*. Nevertheless, an extra contamination of the broilers is noticed during transport and during the slaughtering process, which indicates the importance of hygiene during these steps. The evisceration method and the time of slaughtering (slaughtered as first flock or not) do not have a significant influence on the contamination of the final product.
- *Salmonella* and *Campylobacter* isolates are both showing a considerable antibiotic resistance. Of the *Salmonella* strains 42% were resistant for at least 1 antibiotic, 11% of the strains were resistant for 5 antibiotics. The very high resistance of *S*. Hadar isolates is striking. Of the *Campylobacter* isolates 94% were resistant for at least 1 antibiotic, 2 strains were resistant for 4 and 1 strain for 5 antibiotics.