Assessing the role of sow bile for the exposure of *Salmonella* to humans

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Abstract
We investigated the exposure risk to consumers associated with potential contamination on sow carcasses with bile containing *Salmonella*. A total of 300 samples of bile were taken aseptically from sows on the largest sow abattoir in Denmark. The samples were subjected to selective enrichment followed by a sensitive chromogenic medium (RAPID*Salmonella*) to select for *Salmonella* and other family members. Subsequently, MALDI-TOF was used to identify bacteria appearing on the agar plates at species level. No *Salmonella*-positive samples were found in 300 samples. A simulation model was set up to estimate the number of bile-contaminated carcasses with *Salmonella* leaving the abattoir in one year. Two different scenarios were run: a) assuming the probabilities of bile detection provided by the food business operator or b) provided by the competent authority. The results of simulation showed that the number of bile-contaminated sow carcasses with *Salmonella* was low irrespective of which scenario was run. In conclusion, bile contamination is not a relevant source of *Salmonella* contamination in Danish sows. Still, bile contamination should be prevented as it is resulting in meat waste, because bile contamination is a quality issue.

Introduction
According to the European Food Safety Authority, *Salmonella* is the second most common zoonotic hazard, causing many human cases, and only surpassed by *Campylobacter*. Pig meat is one of the main sources of *Salmonella* (EFSA, 2011). Therefore, many resources in the pig supply chain are spent on reducing the exposure of humans to *Salmonella*. In Denmark, both pre-harvest and post-harvest actions are taken, and this has led to a relatively low human incidence. In 2019, 19.3 new cases of *Salmonella* per 100,000 inhabitants were observed, where 8% were ascribed to nationally produced pig meat (Anon., 2019). On the Danish abattoirs, the focus is on keeping hygiene high and hereby avoiding faecal contamination, which is believed to be the main source of contamination of the carcass.

In 2020, the Danish Competent Authority (CA) raised questions about the role of bile-contamination in the exposure of humans to *Salmonella*. The CA also changed the required handling of bile contamination, so the Food Business Operators (FBO) would not be able to handle these cases alone but would have to await the decision of the CA. This led to the initiation of a study on the risk related to the contamination of sow carcasses for the exposure of consumers to *Salmonella*. The main objective was to estimate the annual number of overlooked carcasses with *Salmonella* due to bile-contamination if the FBO had responsibility for cleaning the carcasses and the CA had the role of verifying procedures.

Materials and methods
In the fall of 2022, 300 bile samples from slaughtered sows were randomly collected at the largest sow abattoir in Denmark. The sampling was distributed over 3 weeks, and during each of these weeks, sampling took place over 2 days to ensure a broad representation of Danish sow herds.
previously developed protocol was used to ensure aseptic collection of the bile to avoid cross-contamination with *Salmonella* from other sources, e.g., the faeces.

After collection, the samples were analysed at the University of Copenhagen. Here, a selective enrichment step was followed by a sensitive chromogenic medium (RAPID’*Salmonella*) to select for and differentiate *Salmonella* from other family members. MALDI-TOF was used to identify bacteria appearing on the agar plates at the species level.

To get a broader picture of the more practical side of meat inspection and abattoir setup, expert opinion was obtained through interviews and questionnaires with personnel working either for the FBO or the CA, as well as the Danish Classification Inspection.

**Statistical methods**

A simulation model was set up to assess the number of sow carcasses with *Salmonella*, due to bile-contamination, leaving the abattoir in 1 year. This exposure risk model contained three variables:

1. Probability of *Salmonella* being present in sow bile,
2. Probability of sow carcasses being contaminated with bile and
3. Estimated proportion of bile-contaminated sow carcasses going unnoticed by the FBO if they had full responsibility for handling bile-contamination.

The data used to inform the parameter for the first variable originated from the 300 sow bile samples as well as the outcome from a similar study on 299 bile samples from finishing pigs, which were also all negative. The data used to inform the parameter for the second variable originated from the Danish meat inspection database, which showed that 5.8% of the sow carcasses are contaminated with bile.

The data to inform the last variable were based on expert opinions from the CA and FBO. The views of the CA and the FBO varied substantially; the FBO were optimistic about their ability to detect the bile-contaminated carcasses, whereas the CA were more pessimistic. Therefore, it was decided to run two scenarios.

**Results**

None of the 300 samples were positive for *Salmonella*. Other Enterobacteriaceae were found, and these consisted mainly of *E. coli* (165/300), followed by *Klebsiella* spp. (8/300).

The FBO-scenario showed that a median of two carcasses with *Salmonella* due to contamination with bile (90% C.I. 0 – 12) would go unnoticed in a production of 281,000 sows in 1 year. Similarly, the CA-scenario showed a median of 12 carcasses (90% C.I. 1 – 57).

**Discussion**

Hence, the exposure risk to *Salmonella* related to bile-contaminated carcasses was found to be negligible. The same conclusion was reached in a similar study in finishing pigs undertaken 1 year ago, which led to a change in the officially required handling of bile contamination on finishing pig carcasses.

The FBO have procedures in place when observing bile contamination due to the yellow colour which creates a quality issue. Handling consists of cutting away the area with discoloration, implying partial condemnation causing an economic loss and an associated negative impact on the climate. Further focus on preventing bile contamination could therefore be beneficial.
To get a better understanding of the amount of meat wasted by trimming of bile contaminated areas, an estimation was made by use of the data reported in association with all slaughterings in Denmark over a 5-month period in 2022. This data is collected in relation to the price the farmer is given for the slaughtered animal (Christensen, 2022). According to the data, the estimated amounts of meat trimmed and discarded meat due to bile contamination corresponded to approximately 670 tonnes during the 5-month period, corresponding to €1.1 million. To reduce the amount of meat wasted, bile contamination should be prevented, where possible. According to responses from the manager of slaughter at one abattoir, it is of paramount importance that the operators, who remove gallbladders, receive correct instruction and training. The abattoir had had success in reducing the prevalence of bile contamination by focusing on the job tasks of these operators: The prevalence of bile contamination plummeted by 50% when the tasks of these operators were narrowed to focusing on the gallbladder removal solely.

New camera-based vision technologies are in the pipeline which could assist in detecting bile contamination effectively to ensure that carcasses with bile contamination do not leave the abattoir before handled effectively (DMRI, 2023). This equipment is developed to detect several different kinds of contamination including faeces and bile. The equipment can detect and show the operator the location of the contamination on the carcass. If a system like this is implemented, an audit-like structure should be developed to ensure corrective actions are carried out when contaminations are registered.

**Conclusion**

No *Salmonella*-positive bile samples were detected out of the 300 collected. According to the simulation exercise, It was concluded that bile contamination on sow carcasses is associated with a negligible exposure risk of *Salmonella* in humans. Bile contamination is a quality issue and should be handled properly by the FBO, and the focus should be on prevention to keep the amount of meat trimmed off low. Bile contamination should be dealt with by the FBO, whereas the CA’s role is to verify the correct procedures are being complied with.

**References**


Christensen, H., 2022. Cheifinspector at the Danish Classification Inspection (Personal communication).
