Hygiene aspects of manure management and antimicrobial resistance in integrated pig-fish farms in Vietnam

This year dr. Dang Thi Thanh Son National Institute of Veterinary Research submitted her PhD thesis and defended it with acclamation at the PhD viva. In this newsletter the most important findings of her study is presented.

1. General objective of the PhD study: To describe hygiene aspects of pig manure management and antimicrobial resistance of fecal indicator bacteria and food safety aspects of integrated pig- fish farms in Vietnam

2. Specific objectives

2.1. To describe hygiene aspect of manure management in pig farms in Northern Vietnam

2.2. To compare the survival of fecal bacterial indicators in clay- covered heaps of pig manure added straw, lime stone and urea

2.3. To study the impact of medicated feed on the development of antimicrobial resistance in fecal bacterial indicators in integrated pig-fish farms

2.4. To compare the level of *E. coli* contamination of fish collected from ponds applying pig manure as fertilizer

Collecting information about pig manure management from households by questionnaire interview (**Paper I**)

Collecting pig manure samples from storage heaps (**Paper II**)

3. **Publications**

3.1. **Paper I:** "A survey of manure management on pig farms in Northern Vietnam".Vu, T.K.V., Tran, M.T., and **Son, T.T.D**. 2007. *Livestock Science*. **112**, 288-297

3.2. **Paper II:** "Survival of fecal indicator bacteria in treated pig manure stored in clay-covered heaps in Vietnam". **Son, T.T.D**., Dung, V.T., Madsen, H., and Dalsgaard, A. 2011. *Veterinary Microbiology*. **152**, 374- 378

3.3. **Paper III:** "Impact of medicated feed on the development of antimicrobial resistant bacteria in integrated pig-fish farms in Vietnam".**Son, T.T.D**., Petersen, A., Dung,V.T, Huong, T.T.C., and Dalsgaard, A. 2011. *Applied Environmental Microbiology***. 77**, 4494- 4498

3.4. **Paper IV:** "*E. coli* contamination of fish raised in integrated pig-fish aquaculture systems in Vietnam". **Son, T.T.D.** and Dalsgaard, A. 2011. Re-submitted to *Journal of Food Protection*, as short communication.

Collecting muscle tissue samples from fish (**Paper IV**)

4. Conclusion, perspectives, and future research needs

The most important findings in the thesis were:

1. Pig production develops rapidly in Vietnam where it causes environmental pollution. Use of untreated pig manure as fertilizer in agriculture and aquaculture is common in rural areas and may be an important source of pathogen transmission. A field survey in Thai Binh and Bac Giang provinces showed that large volumes of untreated pig manure was discharged into rivers, lakes or household gardens. Most farmers are generally aware of the negative environmental impacts and potential health risks that are associated with manure when disposed off in the environment. However, there is little incentive to adopt environmental friendly manure management technologies and practices due to lack of regulation and/or enforcement of existing regulations. So there is a need to improve current pig manure management practices through training of in particular small- scale pig farmers.

2. Most surveyed small-scale pig farming households lacked land for storing manure within the farm perimeter. Thus, such farmers have to transport pig manure and stored it in heaps in the field. Manure heaps are normally covered by a 3-5 cm thick layer of wet mud. Amendments added to the stored manure varies between farms and include straw, ash, lime, superphosphate and urea. In manure heaps added 2% w/w urea ($(NH_2)_2CO$), a hygienic safe and high-value fertilizer was obtained with *E. coli* reductions of about 4 log-10 CFU/g to below the detection limit within two weeks of storage. However, other amendments to the manure heaps were associated with little or slower *E. coli* reductions. Numbers of *Enterococcus* spp. were not reduced in the heaps added urea or other amendments. This indicates possible re-growth and that entercocci are not good indicators of the hygienic quality of stored manure. Future studies should assess cost-benefits as well as hygiene and practical aspects for Vietnamese rural farmers of using ammonia as a sanitizer of pig manure stored in clay-covered heaps.

3. At the integrated pig-fish experimental farm, the development of resistance to NAL and ENR, but not to TET, among *E. coli* and *Enterococcus* spp. isolated from manure and water-sediment samples was significantly associated with the provision of feed containing the

two antimicrobials. The results showed that when pigs were administered feed with antimicrobials, *E. coli* showed significant higher frequencies of resistance towards NAL and ENR as compared to when the feed did not contain any antimicrobials. For NAL, 82% and 70% of *E. coli* strains isolated during months 2 and 4 were resistant as compared with 38% and 32% resistant *E. coli* found in months 1 and 3, respectively ($P< 0.001$). A similar trend was seen for ENR where the frequency of *E. coli* strains resistant to ENR was 75% in month 2 and 43% in month 4 as compared with 32% and 17% for months 1 and 3, respectively ($P<0.01$). The frequency of ENR–resistant *Enterococcus* spp. was lower when compared with *E. coli*. Although the prevalence of ENR resistance in month 3 was lower than in period 2 and 4 ($p<0.001$), there was not a clear association between the development of resistance and provision of feed containing ENR. TET resistance was high (> 95%) in both bacterial indicators throughout the study period which indicates that TET-resistant *E. coli* and *Enterococcus* spp. were transmitted to the piglets before the initiation of the experiment. PCR-based identification showed similar relative occurrences of *E. faecium*, *E. faecalis*, and other *Enterococcus* spp. in manure and water/ sediment samples, suggesting that *Enterococcus* spp. isolated in the ponds mainly originated from the pig manure.

4. Fishponds are widespread in rural areas in Vietnam where the culture of fish is often integrated in so-called VAC systems (Vuon- garden; Ao- pond; Chuong- pigsty). The manure and other livestock wastes are discharged directly the pond where the nutrients support growth of algae and other organisms eaten by the different fish species stocked. Three different fish species collected from ponds that used pig manure contained significantly higher numbers of *E. coli* in the gut content than did gut content from fish in ponds where pig manure was not applied. In ponds with no pig manure applied the mean log-10 concentrations of *E. coli* found in silver carp, grass carp, and rohu were 3.32, 2.63, and 3.04 (CFU/g), respectively. Where as in ponds applied manure, numbers of *E. coli* in silver carp, grass carp, and rohu were 4.75, 5.25, and 5.07 (CFU/g), respectively. Fish grown in both pig manure-fed and control fishponds were of very good quality at harvest with no *E. coli* found in samples of muscle tissue that were collected through procedures that avoided cross-contamination. Further studies are needed to determine the food safety of fish produced in integrated livestock-fish systems, including hazards represented by fishborne zoonotic parasites and other pathogens as well as accumulation of toxic chemicals and antimicrobial resistance bacteria and genes.

Researches need:

1. Develop in close collaboration with farmers improved, environmental sustainable, and cost-effective livestock waste management practices with focus on large- scale pig farms.

2. Determine the pathogen die-off and uptake of nutrients in fishponds receiving biogas effluent.

3. Assess human health and environmental hazards associated with integrated livestock-fish production systems with focus on pathogen transmission and chemical residues.

4. Conduct field-based and scaled experiments to determine the sanitizing effect of adding ammonia in clay-covered manure heaps as documented through pathogen die-off.

5. Assess potential environmental and human health risks associated with development and spread of antimicrobial resistance in integrated livestock-fish production systems.

6. Study how fish of acceptable food quality can be produced in integrated livestock-fish systems with focus on prevention and control of pathogen transmission, emergence of antimicrobial resistance and accumulation of chemical residues.