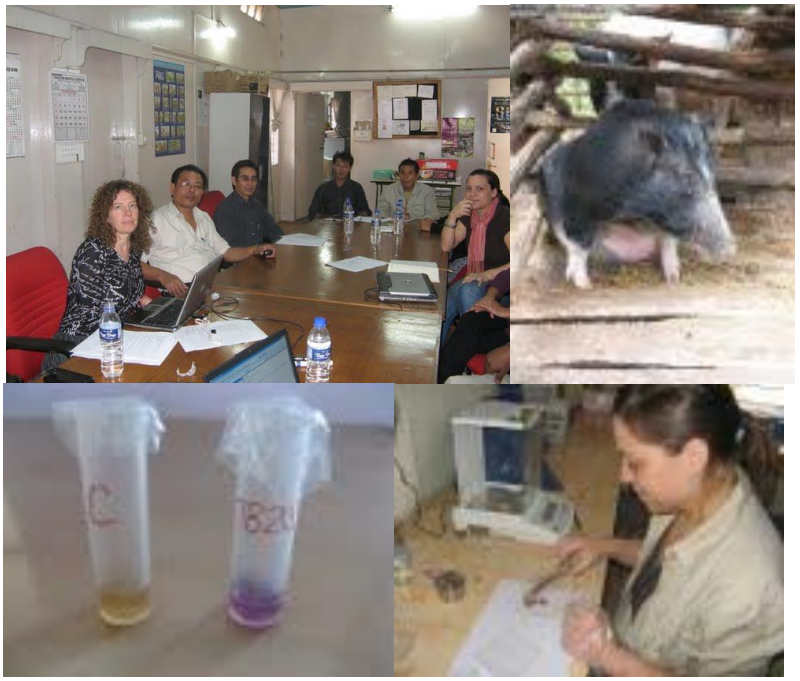
# Disease risk assessment in pig value chains:

## A constructive study in Nagaland



Delia Grace
Anna Fahrion
Lanu Jamir
Sonuwara Begum

December 2009

# Disease risk assessment in pig value chain in Nagaland

**Background**

Most poor people buy and sell animal products in the informal markets where there is little safety and quality regulation. Studies on livestock products in these markets typically find high levels of pathogens and other hazards such as toxins or drug residues. These not only impose a heavy burden of avoidable sickness and death to consumers, but constrain producer access to higher value markets where standards prevail. Animal disease, including zoonoses, is also a key constraint to increasing production and productivity.

In recent years, risk-based approaches have become the gold standard for assuring food-safety (Codex Alimentarius framework) and also the basis for international trade in animal products (OIE framework). Risk assessment offers a science-based, structured, transparent method for answering the key concerns of policy makers and public alike: Is it safe? Is it a big and important risk? What efforts are appropriate to reduce the risk? Risk management uses pathway approaches (from stable to table) and probabilistic methods (decision trees, scenarios, Monte Carlo modelling) to identify those critical points where control can be effectively applied to remove or minimise risk. Risk communication is the iterative process of communicating risk to those affected by it and incorporating their feedback into risk assessment and management.

In order to apply risk assessment usefully, a priority list of problems is needed. There are several hundred food-borne hazards and two to three times as many diseases of pigs. It appears that in most cases, a small number of hazards cause the great majority of harm However, these 'vital few' hazards vary from case to case. Risk ranking involves a systematic and screening process to reduce the list of all hazards to a smaller number of 'priority hazards' which are most likely to be problematic in any given case. Unlike risk assessment there are no standard or consensus methodologies, and currently one of the most active areas of non non-laboratory research is "risk ranking[1]" risk assessment techniques as a means of finding more objective comparisons of risks to aid in the allocation of scarce food safety resources.

A risk profile can then be developed for priority hazards. This is a broad and qualitative summary of relevant information on a specific food safety issue or animal disease. It can contain information on: the hazard, its impact on human and/or animal health, the population affected, incidence and prevalence, epidemiology of transmission, stakeholder concerns, relative importance of the hazard, and options for management, etc. The major output of the risk profile is the recommendations whether or not to further address the problem and the recommendation to whether or not to commission risk assessments

Risk assessment as a structured systematic process to support food safety risk management has been largely driven by the international community. The Codex Alimentarius Commission (CAC)

[1] Also known as Hazard Ranking, Risk Attribution, Risk Risk-Based Priority Setting, Comparative Risk Assessment (CRA), Maintaining a "Risk Register"

has developed a four stage method of microbial risk assessment. This is the most credible way of assessing food safety risks, although its cost and complexity imply adaptation is needed for application to informal markets.

1. Hazard identification: provides a summary of the pathogen/toxin and disease caused.
2. Exposure assessment: characterizes the 'stable to table' path
3. Hazard characterisation: describes dose response and adverse impacts
4. Risk assessment: gives a synthesis of adverse effects and their likelihoods

A study by ILRI[2] indicates that "in Nagaland, as elsewhere in the North Eastern Region, there is little or no formal infrastructure for slaughter of pigs or display of pork, which raises concerns about public health issues related to food safety". Though these risks to human and livestock health are real and could have major impacts, the lack of public awareness is found to be one of the major issues. To start with, the health risks (of both humans and livestock) are to be assessed, followed by capacity building of the actors concerned to mitigate the risks.

Against this background, ILRI through its ELKS (Enhancing Livelihoods through Livestock Knowledge Systems) programme, supported by the Navajbai Ratan Tata Trust (NRTT) has decided to conduct a study **"Disease Risk Assessment in Pig value chain"** in association with the Government of Nagaland (DV&AH) and NEPED, one of NRTT partners in North East India.

## Materials and Methods

### 1a. Setting, Collaborations

Working base was the city of Kohima, state capital of Nagaland 2nd largest town (after commercial centre Dimapur). We had the kind allowance to use the facilities of the "Office of the district Veterinary and Animal Health Officer" in Kohima for lab work and storage of samples. Additionally, the animal health department provided us manpower. Office facilities and a meeting room were available at the headquarters of NEPED (Nagaland Empowerment of People through Economic Development) which constitutes another local collaboration partner of ILRI.

The district of Kohima is located in the south of the state and includes an area of 927 sq.kms with a population of about 315 000[3], representing about 1/6 of Nagaland's population. All villages included in the study are located in this disctrict.

### 1b. Study period

The core study period was in November 2009. It started with a 3-day introductory workshop on nov 5th -7th for members of all participating collaborators (Nagaland Animal Health Department, ILRI, NEPED) including field staff for sampling, laboratory work, participatory risk assessment and questionnaires/check lists. In parallel, the laboratory was set up and some trial samples

[2] Deka, R. and Thorpe, W. 2008. Nagaland's pig sub sector – current status, constraints and opportunities
[3] Nayak, P (ed.): Growth and human Development in North-East India, Oxford University Press (2010)

taken. In the following period until end nov 2009, the major part of the sampling and investigating work was carried out.

2. Identification of pork meat chains to investigate in Kohima district, Nagaland

In Kohima district, there were two basic chain structures of the pig market identified, though the demand for traceability from farm to fork could not completely be fulfilled in both. In fig.1 and 2, chain elements which could be taken into account are circled.
These are the two different chains identified and taken into account for Nagaland in the study:

1. Rural pork meat production chain: Rearing/Fattening of self breeded or -more often- bought pigs from weaning age on, slaughter and consumption in the same village. This happens most of the time with small backyard animal husbandry, most frequently with a size of 1-5 pigs. In this chain, (producer=) fattener = slaughterer =butcher = consumer; there's not a real "chain".

2. Urban Kohima pork meat production chain: Arrival of ready-fattened pigs, most of the time via Dimapur from outside the state, sold to urban slaughter-places in Kohima. Sale and transport of carcasses to town butchers in Kohima, where meat is sold to consumers (see fig.2)

1. Checklists/questionnaires

Checklists and questionnaires were developed for the use on different pork chain levels:

a) Producer (farmer) observation check list
b) Slaughter check list and questionnaire
c) Transporter interview
d) Butcher (retail) check list
e) Butcher (retail) questionnaire
f) Consumer questionnaire

In discussion with the local collaborators, the mentioned tools were adapted in respect of the country's social particularities and conversational habits[4].
Questionnaires were filled out by collaborators in a direct interview with the respective target person; check lists were filled out on site but had most often to be complemented by direct interrogation as well (see also: Instruments (check lists, questionnaires) themselves).

2. Participatory risk assessment (PRA):

The PRA was developed to work on production level, with the farmers. The concept included *proportional piling* as a tool to assess the relation of pigs born in, or bought to, the village, and those removed by slaughter or death, or sold outside the village. The proportional piling was carried out with beans representing the pigs and a circle on paper depicting the village.
The PRA additionally contained sections to discuss important diseases and typical syndromes of diseases with the help of a matrix promoting an *ethno-veterinary investigation* of health problems in pigs, *describing* and *ranking*.

[4] e.g. the consumer questionnaire included some questions about sickness that would have been extremely rude to ask in the Vietnamese conversation conventions but did not hurt Naga people's sentiments. So, in a parallel study in Vietnam these respective questions were replaced or omitted.

A *focus group discussion* was aimed to estimate what the PRA group represented in relation to the whole village population.

A group of 5-10 farmers per village was assembled (through local contacts) and the PRA was carried out in a 2-3 hours period, if possible sitting in a circle allowing active participation (e.g. in proportional piling). One project staff served as a protocolist and another one as facilitator, if possible a third one (from local staff) as a translator. Often, these roles stayed rather flexible depending on the flow of discussion and due to changing project staff taking part in the activities.

Following the PRA session, village community members (most often farmers or other representatives that had taken part in the PRA) took project staff on a tour through the village and to visit some (6) farms and stables, where producer observation check lists were filled out on-site. Consumer interviews were done whenever possible.

3. Laboratory diagnostics:

Laboratory tests were carried out to estimate pathogenic burden of pig/meat samples on two important control points of the chain: fresh slaughtered pigs on the slaughterhouse level and meat samples on the butcher/retailer level.

From the fresh slaughtered pigs, infectious burden was evaluated by taking blood and faecal samples and carrying out a tongue palpation; on butcher level, meat samples were tested for contamination in the process of transport and cutting up.

- *Blood samples* were taken from vena cava blood during the slaughtering process when liver and intestine were cut out of the carcass ; it was taken in a 5 ml or 10 ml sterile syringe out of the abdominal cavity and stored in 7 ml sterile plastic tubes without additives. In the following, it was stored on ice and allowed to clot for approx. 12 hrs at 4°C followed by centrifugation at ~1200 rpm for 10 min. The serum was pipetted into sterile 2 ml tubes and frozen at -18°C until utilization in the Brucella serologic test as described in the manufacturer´s instructions.[5]

- *Faecal samples* were taken during slaughter process out of the rectal part of the intestine when it had been taken out of the abdominal cavity and before it was processed further. Approx. 10 g of faeces were put in a plastic bag which was closed and stored in a cool box and later in the fridgerator. Within the 48 hours following the sampling, the faeces underwent a sedimentation-floatation process as described in literature and were observed under the microscope for eggs/oocysts of different protozoa and helminth species and assessed following a semi quantitative scale[3].

- The *lingual palpation* for cysticercosis was carried out by project veterinarians *in situ* in fresh slaughtered animals ante rigor mortis using a plastic glove[3].

[5] see SOPs "Stepwise lab instruction sheets for pathogen diagnostic tests, pig risk assessment study" (A. Fahrion)

- *Meat samples* (approx. 25 g each) were bought or collected from butchers´ stalls by local collaborators. They were stored individually in plasic bags or containers on ice and brought as soon as possible (within one hour) to the laboratory were they were processed immediately to give an account of real bacterial colonisation at sales point. For this purpose, meat was processed under sterile conditions following the respective instructions[3].

The following tests were carried out with the above described material following the respective manufacturer´s instructions:

| **Pathogen/Disease tested for** | **Name of test** | **Test principle** | **Material used** |
|---|---|---|---|
| Total aerobic bacteria | HiTouch Aerobic Count Flexi Plate (Himedia, India) | Agar plate | Homogenized suspension (meat sample + peptone water) |
| Enterobacteriaceae | HiTouch E.Coli/Coliform Count Flexi Plate (Himedia, India) | Agar plate | Homogenized suspension (meat sample + peptone water) |
| Staphylococcus aureus | Petrifilm™ Staph Express Count Plate (3M, St. Paul, MN, USA) | Selective growth medium (petrifilm) | Homogenized suspension (meat sample + peptone water) |
| Listeria | Petrifilm™ Environmental Listeria Plate (3M, St. Paul, MN, USA) | Selective growth medium (petrifilm) | Homogenized suspension (meat sample + peptone water) |
| Brucella suis | Brucella IgG Flow Assay (KIT Biomedical Research, Royal Tropical Institute, Amsterdam, NL) | Immunochromatographic lateral flow test | Serum |
| Cysticercosis | Manual tongue palpation | Visual and palpatory examination for Cysticercus cellulosae | Tongue *in situ* of fresh dead slaughter pigs |
| Intestinal parasites | Sedimentation with water, Floatation with 33% $ZnSO_4$ solution | Sedimentation – Floatation tests | Faeces |
| Antibiotic residues | Premi®Test B.V. (DSM, Heerlen, NL) | Inhibitation test with colorimetric indicator using *Bacillus stearothermophilus* | Meat juice |

3. Sample numbers

**1. Rural pork meat production chain:**

On *village level*, convenience sampling had to be practiced for the choice of participating villages, as we wanted to stick to the trace back approach as far as possible and had to rely on local collaborators to find out about village slaughter dates and connect with the village population. So the villages chosen were those some collaborator had connections to (own

village, family member's village, NEPED project village) and which ideally slaughtered a pig during the study period.
As an approach, it was fixed that the following objectives should be obtained:

→ **Total objective villages**:
- 5 village slaughtered pig samples : blood, faeces, lingual palpation
- 10 PRAs
- 60 Producer Observation check lists
- 60 Village Consumer Questionnaires

**2. Urban Kohima pork meat production chain:**
Four representative *slaughterhouses* of Kohima town were chosen, among them the two

| **Village** | **No. to visit** | **Samples to obtain/ information to gain *per village*** |
|---|---|---|
| Villages killing a pig | 5 | Blood sample<br>Faeces sample<br>Lingual palpation<br>PRA<br>6 Producer Observation Check lists<br>6 Consumer questionnaires |
| NEPED project villages | 5 | PRA<br>6 Producer Observation Check lists<br>6 Consumer questionnaires |

largest ones

- "Don Bosco" and "Newmarket", both with slaughter volumes around 20 pigs per day and two with less important business volume:

- "Bridge/Chandmarie" (slaughter volume per day: 3-7 pigs) with smaller slaughter facilities where the slaughter is done following direct demand and direct retail sale to consumers (omits carcass transport step)

- "High School" (slaughter volume per day about 10 pigs) which is a newer enterprise and possesses a considerable infrastructure (like electricity, running water supply and concrete floor) compared to the others.[6]

The Objectives for slaughterhouse sampling were:

| **Establishment/person** | **No. to visit** | **Samples to obtain/ information to gain each** |
|---|---|---|
| Large slaughterhouses | 2 | Slaughter check list and questionnaire<br>30 blood samples[7] |

[6] For more detailed descriptions of the slaughter establishments, see separate document "PigRA Naga_ObservationSlaughter" (A. Fahrion)

| | | |
|---|---|---|
| | | 30 fecal samples<br>30 lingual palpations |
| Small slaughterhouse (High School) | 1 | Slaughter check list and questionnaire<br>15 blood samples[5]<br>15 fecal samples<br>15 lingual palpations |
| Transporters | at 3 slaughter points as listed above | Transporter Interviews, each:<br>2 Traders coming from Diampur<br>3 Traders going to Kohima |
| Slaughterhouse and sales point (Bridge/Chandmarie) | 1 | Slaughter check list and questionnaire<br>15 blood samples[5]<br>15 fecal samples<br>15 lingual palpations<br>10 samples meat |

**→ Total Objective Urban Slaughterhouses:**

- 4 Slaughter check lists and questionnaires
- 15 transporter interviews
- 90 slaughter pig samples (blood, faeces, lingual palpation)
- 10 meat samples from slaughter-sales point

Concerning *butchers* and *consumers*, a choice of 25 Kohima town butchers was made.

| **Butchers to sample** | **Samples to obtain/ information to gain *each*** |
|---|---|
| 25 | Butcher checklist<br>Butcher questionnaire<br>3 meat samples : early, noon, late[8]<br>6 consumer questionnaires (customers of the respective butcher) |

**→ Total Objective Urban meat retailers and customers:**

- 25 butcher check lists
- 25 butcher questionnaires
- 75 meat samples
- 150 consumer questionnaires

[7] All mentioned blood and faecal samples and lingual palpations had to be distributed on 3-4 different sampling days to guarantee the involvement of diverse batches

[8] To be taken at 6-8 am, 8 am - 1 pm and after 1 pm, respectively.

**Initial findings from the Pork Risk Assessment in Nagaland**

Key results

- Some important hazards are present at unacceptably high levels in pork meat in Kohima and surrounds
- Village slaughtered pigs tend to have better bacteriological quality
- Delayed sale of meat (after 9.30 am) is a key cause of poor quality
- If one microbiological quality indicator is poor, then the others are also likely to be poor
- Butchers tend to have consistently good or poor microbiological results suggesting training may be useful to improve poor performers

**Hazard characterisation**

What hazards are present in pork? What harm can it they do to people? How high are the levels of hazards?

| **Hazard or indicator** | **Hazard characterisation** | **Prevalence** | **Unacceptable at point of sale** |
|---|---|---|---|
| Total plate counts | A general indicator of the quality and safety of pork | | 22.5% |
| Listeria monocytogenes | One of the big 5 food-borne diseases.<br>Causes serious disease in vulnerable groups such as the elderly and pregnant women | 33% | 0% |
| Coliforms | An indicator that pork has been contaminated by faeces.<br>Many food-borne disease are passed via faeces. | 92% | 40% |
| Staphyococcus aureus | One of the big 10 food-borne diseases.<br>Bacteria produce toxins which are not destroyed by cooking.<br>A good indicator of bad-handling | 93% | 47% |
| Cysticercosis | A serious neglected zoonoses.<br>A major cause of epilepsy | 9% | yes |
| Brucella suis | A serious zoonoses<br>Production disease in pigs – causes still birth, abortion infertility | 6% | ? |
| Antibiotic residues | Can cause reactions in sensitive people<br>Fosters development of resistance in bacteria affecting humans.<br>Many are not destroyed by cooking | 5% | ? |

**Which is safer: village killed or town killed pigs?**

| | Village | Town | Conclusion | p |
|---|---|---|---|---|
| Unsafe coliforms | 20% | 80% | Town worse | 0.004 |
| Unsafe staph | 20% | 48% | No difference | 0.219 |
| Antibiotic residues | 20% | 4% | Country worse | 0.087 |
| Brucella suis present | 0% | 6% | No difference | 0.565 |
| Cysticercosis present | 20% | 8% | No difference | 0.365 |

(test chi 2 adjusted for clustering on butcher)

Town slaughtered pigs have higher levels of coliforms an indication of contamination of the carcass with faeces. Country slaughtered pigs have more antibiotic residues. Other differences were not significant.

**Which is safer: self slaughter or abattoir slaughter**

| | Self | Abattoir | | |
|---|---|---|---|---|
| Total plate count | 3790 | 7620 | No difference. | 0.2218 |
| Unsafe coliforms | 84% | 90% | No difference | 0.245 |
| Unsafe staph | 10% | 53% | Self is better | 0.010 |
| Antibiotic residues | 10% | 3% | No difference | 0.243 |

Among the town butchers, most got their meat from one of 3 slaughterhouses, however, one slaughtered his pigs when needed. His meat had lower levels of staph aureus, which is an indicator of unhygienic handling as well as an important cause of food-borne disease.

**Is there a relation between different hazards?**

| | *Poor TPC* | *Unacceptable coli* | *Unsafe staph* | *Antibiotic residues* | *Listeria present* |
|---|---|---|---|---|---|
| Poor TPC | 1.00 | | | | |
| Unacceptable coli | 0.56 | 1.00 | | | |
| Unsafe staph | 0.45 | 0.40 | 1.00 | | |
| Antibiotic residues | 0.02 | -0.06 | -0.21 | 1.00 | |
| Listeria present | 0.04 | -0.01 | 0.21 | -0.16 | 1.00 |

Calculating correlation coefficients showed a strong relation between poor total plate counts, coliform counts and staphylococcus aureus, but not with antibiotic residues or Listeria.

**Does purchase time affect the quality of meat?**

There is a strong effect of purchase time on quality of meat. Early sampled meat has the lowest total plate count and late sampled meat the highest. The difference between early sampled and mid sampled and mid sampled and late sampled are highly significant (p=0.009 and p=0.005 respectively). However the difference between mid sampled and late sampled is not significant.

| sample time | Mean TPC | Time of sample | Freq. |
|---|---|---|---|
| Early | 2940 | 7.00am – 9.30am | 25 |
| Mid | 8778 | 10.00 am- 12.30 | 30 |
| Late | 9138 | 1.00 pm- 3.00 pm | 29 |

(One way ANOVA following square root transformation with Bonferroni comparisions)

Samples with unacceptable coliform levels were also significantly fewer for early samples (p=0.025) as were samples with listeria present (p=0.056). However, there was no significant difference for staph contamination or for presence of antibiotic residues.

**Do some butchers consistently produce meat of higher standards over time?**

Because we only took 3 samples from each butcher and these were taken at different times it is difficult to measure the consistency of quality between samples. However, even with this small sample size, there was some evidence that samples taken from the same butcher were more similar than those taken from other butchers (i.e. some butchers are consistently better and some are consistently worse).

Intracluster correlation coefficients are a measure of similarity between members of a group. Positive values suggest members of a group are more like other members of the same group than members of other groups. Negative values suggest the reverse. All intracluster coefficients for butchers were positive and they varied from negligible to high indicating consistency among butchers.

| Quality measure | ICC | ICC 95% confidence interval | interpretation |
|---|---|---|---|
| Unsafe coliforms | 0.27 | 0.02-0.51 | high |
| Total plate count | 0.05 | 0.00-0.28 | moderate |
| Antibiotic residue | 0.10 | 0.00-0.34 | moderate |
| Unsafe staph | 0.002 | 0.000-0.230 | small |
| Listeria present | 0.00 | 0.00-0.23 | negligible |

**Is there any difference between slaughterhouses in presence of pathogens?**

In general there was no difference between slaughterhouses in presence of pathogens. However, cysticercosis, an important zoonoses, was significantly higher in slaughter house 2.

| | **S1** | **S2** | **S3** | **S4** | **p** | |
|---|---|---|---|---|---|---|
| Br. suis | 13% | 0% | 6% | 0% | 0.49 | No difference |
| Cysticercosis | 13 | 50 | 0 | 8 | 0.01 | Difference |
| Oocysts | 55 | 67 | 41 | 50 | 0.61 | No |
| strongyloides | 3 | 0 | 0 | 8 | | No |
| Strongyles | 13 | 17 | 10 | 8 | | No |
| Ascarids | 10 | 17 | 0 | 0 | | No |
| Trematodes | 26 | 17 | 17 | 33 | | No |

There was no significant difference between town-slaughtered and village-slaughtered pigs for any of the 7 pathogens. However, the sample size for village pigs was probably too small to detect differences even if present.

Interesting preliminary findings include:

- Pork and meat consumption are both high
- Gastrointestinal disease seems to be common: in most households one person has been sick in the last 2-8 weeks.
- A majority of customers have concerns about the quality of pork meat
- Cysticercosis- infected meat is common: around half consumers have seen it in the last year
- There is a high risk of cross-contamination during food preparation: that is the transferring of bacteria from pork to other food or surfaces
- Consumers have relatively good cleaning practices
- Consumers have poor disinfection practices
- Most pork is cooked thoroughly before eating, reducing the chance of exposure to some (but not all) hazards
- A minority of consumers taste smoked, uncooked pork (a risk factor)