

Tracing back to Sources of MAIC Using Farm records and Lab Techniques

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Summary

For the implementation of tracing back systems (particular agents, unwanted observation and other), detection techniques should be available. For MAIC, a step-by-step approach was established, combining visual observation, lab based PCR-identification, tracing back to the farm of origin and finally the search for potential ports of entry:

- Recording of observations as part of internal or veterinary recording system
- Using a PCR based identification procedure, the agent could be identified
- Based on the ID of the farm, farms at risk could be traced back
- Investigations for ports of entry on farm level may follow.

Introduction

Complexity of food chains requires complex detection-, verification- and inspection systems, all of them must get organised in such a way, that "failures" or "unwanted observations" of any kind are detected wherever along the lines. Only then, corrective measures can be taken.

All such unwanted items require techniques adapted to detection and/or verification of their presence or absence. Prior to implementation, techniques should be assessed with respect to their efficacy (sensitivity and specificity), for their applicability at the position intended, the availability of personnel skills and (if needed) the lab capacity.

For food animals, ante and post mortem inspection still relies on pathological and clinical observation, which has been done since the beginning of the last century in Europe and elsewhere. Basic principle was the indication of pathological observations for a disease or the presence of an agent, which may cause this lesion. Still today, ante and post mortem inspection rely on such indicative values.

Amendment of Statutory Meat Inspection in the EU: From its origin, cutting of the mandibular lymph nodes (LN) was intended to detect tuberculosis in both, cattle and swine, and for both categories, it is still mandatory: For cattle to detect bovine Tuberculosis, for swine, to detect abscesses possibly caused by agents of the Mycobacterium avium intracellulare Complex (MAIC, Table 1). Evidence for human relevance of MAIC is available (immunocompromised persons and children). Also found in LN abscesses is *Rhodococcus equi*, which should be considered as of risk for humans, too (1).

Table 1: *M. avium* and *M. intracellulare* (2)

Family	Mycobacteriaceae
Genus	Mycobacterium
Species	Mycobacterium avium
	ssp. avium
	ssp. paratuberculosis
	ssp. silvaticum
	ssp. hominissuis
Species	Mycobacterium intracellulare

However, visual inspection is not reliable enough, with respect to sensitivity as well as specificity, and abscesses indicating MAIC are supposed to be notoriously underreported.

As abscesses are not indicative for MAIC, microbiological confirmation is desirable, but the slow microbiological detection procedure for members of this family asks for more rapid techniques.

In addition, cutting of LN carries the risk of contamination, in particular, if other tissues (e.g., tonsils) are going to be cut simultaneously. A high percentage of tonsils and gut lymph nodes of finisher pigs may harbour *Salmonella*, *Campylobacter* and to a smaller extent *Yersinia* or *Listeria* (3).

So, replacement or improvement of an inaccurate (from the information point of view) and risky (from the contamination point of view) inspection procedure with more reliable techniques is required. It was the aim of this study to implement a daily practice system of

- Detecting abscesses, simultaneously tracing back to the respective farm of origin
- Laboratory based confirmation of the agent from suspicious LN
- Feeding back from the suspicious amount of lesions to the farm
- Sampling at the farm, detecting and closing possible ports of entry

Material and Methods

Information from post mortem inspection: Post mortem data (2005 to 2009) of the veterinary services and recorded via inspection terminals were scrutinised for lesions possibly indicating MAIC infections. Data stem from two cooperating farmer associations (fattening pigs), all finishers were slaughtered at one single abattoir.

Data of interest were transferred into a separate table, calculation was done using PASW for Windows: Identification number of the farms, number of animals shipped from these sites and the observations from both, cutting mandibular LN and palpating the gut LN.

Lab data: Sampling and techniques used: In addition, in the year 2007 LN samples with visible and suspicious lesions from finishers were taken during meat inspection from both locations, mandibular and gut LN at the same abattoir.

44 LN with lesions (of them, 11 from the guts) were processed the day after collecting. From these, samples were taken from visually unaffected tissue and in parallel from an abscess directly. Tissue preparation was done as follows:

- Homogenisation with a Retsch- Mixer Mill (MM 2000, Manufacturer Retsch, Germany) and using a one-way stainless steel grinding ball
- DNA- extraction with the aid of a commercial kit (High Pure Template Preparation Kit, Roche) with a few adaptations (4)
- PCR on a Thermocycler Trio (Biometra, Göttingen, Germany) with the primer- pair AV 6/7 (5). The protocol was slightly re-arranged.
- Nested PCR with several variations in the basic protocol was done with the primers AVNF (5'-cga ccg ccg gga cct aac g) and AVNR (5'-gcg ccg acg acc acc aca t)
- Gel-Electrophoresis and documentation with INTAS digital Video- System.

Results

Results of post mortem inspection: Farms were arranged in a list according to the number of suspicious lesions in the mandibular- or gut LN. For every year and both inspection sites, 4 farms with the highest number of suspicious lesions were identified, i.e. in total 20 farms for the mandibular lesion and another 20 farms for the lesions located in the gut lymph nodes. 5 farms appeared 2 times (Table 2):

Table 2: Farm of Origin being Suspicious for MAIC

Farm 235	Guts 2005 / Guts 2009
Farm 187	Guts 2008 / Guts 2009
Farm 91	Mandib. 2005 / Mandib. 2007
Farm 104	Mandib. 2008 / Guts 2008
Farm 7	Mandib. 2006 / Guts 2008

Lab results (Identification and detection techniques): Most lymph nodes examined stem from the mandibular LN (after cutting). Irrespective of the matrix, 28 out of 44 LN were positive for MAIC, MAIC was not obtained from 16 LN.

Positive LN: 28 out of 44

Negative LN: 16 out of 44

Positive results were obtained from tissue without any visible lesion, too (Table 3). However, it should be noticed, that all LN were affected with suspicious lesions. Results indicate, that a PCR based procedure works despite the presence of tissue. However, sensitivity has not yet been assessed.

Table 3: MAIC from 27 Tissue Pairs (Identical LN): Tissue with and without Visible Abscesses

no lesion visible	from abscess lesion	Pairs
MAIC positive	MAIC negative	1
MAIC negative	MAIC positive	10
MAIC positive	MAIC positive	7
MAIC negative	MAIC negative	9

Discussion

The food chain covers the total production sequence, i.e., the whole life of animals, from incoming goods to the farm up to the animals being shipped for slaughter. This MAIC- inspection approach combines daily observation, lab based examination, tracing back and search for ports of entry. It is based on daily practice in a commercial food chain (fattening pigs) in Northern Germany. In detail:

Observation during post mortem inspection:

- Even under the assumption of underreporting and low detection sensitivity, our results indicate, that farms at risk may be detected with the veterinary service inspection routine. However, the risk of contamination remains.
- Visual observation depends on personal inspection capability. Microabscesses must be expected to be overseen, lab based techniques are needed.

Development of lab procedures:

- Here, a PCR was used, tissue background noise was overcome with some modifications in DNA extraction and PCR protocols. Yet, sensitivity has not been investigated.
- LN examined here, did not belong to animals from the farms: In this part of the project, the lab protocol as such was intended.

Tracing back to the farm of origin and possible re-arrangements (biosecurity measures):

- Using the total of observation records over several years from the farms with an identity number, shipments with a high burden of MAIC suspicion were identified, opening the option of tracing back. So, the farm was open for consideration, too.
- Based on this, incoming goods and biosecurity measures on a farm with a high number of pigs with suspicious lesions may be scrutinised in order to eliminate the initial port of entry (not yet done).

An assessment of MAIC with respect to human health remains still open, consequently, inclusion of the MAIC agent into surveillance systems is still undecided and up to risk assessment. Meanwhile, collection of data including fine-tuning of detection systems should be already the issue of the day.

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