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Assessing the relationship between farming practices, laboratory analyses and post-mortem findings: a case study in pig fattening

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Summary

European Union legislation on animal production associated with food safety requires the collection and management of information and data about the farm, the herd and the individual animal. This paper describes the technical steps of the generation, collection and interpretation of data from 296 pig-fattening farms, belonging to two farming associations and using indoor production systems (56 management parameters). The paper also describes post-mortem findings and the results of enzyme-linked immunosorbent assays (ELISA) for antibodies to salmonellae, *Trichinella* spp. and *Yersinia* spp. A total of nearly 30 million data points were collected and analysed for this study.

The results of the ELISA were negative for *Trichinella* spp.; for salmonellae and *Yersinia* spp., both negative and positive results were obtained. Analysis of the farm management parameters showed no significant differences; therefore, the cut-off levels for salmonellae and *Yersinia* spp. were increased, in order to identify farms with a greater hygiene burden. Post-mortem findings possibly related to 'farm hygiene' were used in the analysis. As a result, three farms with particular management decisions were identified as potentially having contributed to the high burden of pathogens detected using ELISA.

A relationship between laboratory results and farm management parameters assessed from yes/no answers could not be established in this study without further work on the available data set.

Keywords

Enzyme-linked immunosorbent assay – Food safety – Meat inspection – Pork – Risk management – Serological examination – Zoonoses.

Introduction

The pork food chain

For food of animal origin, the food chain consists of primary, secondary and tertiary production. Primary pig production includes sub-stages such as breeding, multiplying and finishing. For breeding, sows are kept on multiplying farms (1), whereas multiplying and fattening can take place on one farm or on separate farms (2). The weaning age is approximately four weeks, with a live weight of 6 kg to 9 kg (3). Pigs are then identified with ear tags or tattoos on a herd basis (4), allowing the animals to be traced back to the farm of origin (5). This method is used as part of the traceability system to assist in the management of animal diseases (6).

At 25 kg to 30 kg, animals enter the finishing farms, where they are kept until reaching 110 kg to 120 kg live weight (1, 3). In Germany, the life span of a fattened pig is approximately 180 to 220 days.

Transport connects primary production and slaughter (secondary production) at the abattoir. Lairage (enclosure) is needed for logistic reasons and for the animals to recover from the stress of transport. Slaughter involves stunning and bleeding, followed by scalding and de-hairing, opening, evisceration and splitting. Finally, carcasses and by-products are chilled, before distribution or further processing (tertiary production).

Pork is an important vector of pathogenic agents causing foodborne disease (7). A total of 35 agents of concern have been identified: parasites (12 agents), bacteria (14 agents) and viruses (9 agents), including *Yersinia enterocolitica*, *Campylobacter* spp., *Erysipelothrix rhusiopathiae* and *Toxoplasma gondii* (7, 8, 9, 10). The agents most frequently involved in foodborne diseases from pork are salmonellae, *Y. enterocolitica* and *Campylobacter* spp. (8). In 2006 and 2008, in Germany, salmonellae were found most frequently in fresh pork and poultry meat (7, 11), with *Yersinia* spp. mostly occurring in pig meat (7).

In the European Union (EU), 231 cases of human trichinosis came to the attention of the authorities in 2006 (7). However, almost no infections were reported from conventional pig farms: between 1999 and 2003, *Trichinella* spp. were detected in 0% to 0.000002% of such farms in Germany (12). In contrast, an endemic cycle exists in wild boar (13).

Only limited data are available on the prevalence of *Yersinia* spp. on German pig-fattening farms. In a recent study of samples from farms in north-western Germany, nearly all had animals that were antibody-positive, with prevalences ranging from 57% to 95% (14), and, in a comparative study on outdoor pig holdings, nearly all animals tested positive for antibodies against *Yersinia* spp. (15).

The ‘hygiene package’ of the European Union

The food chain, as described in Reg. (EC) No. 178/2002 (16), brings together aspects of the production line from primary production (including feed and animal transport) through to secondary production (slaughter and processing) and distribution.

Information to be collected from the food chain

For every animal going to slaughter in the EU, irrespective of the inspection procedure, ‘food-chain information’ is required (5):

– the status of the herd of provenance or region

– the health status of the animals

– the veterinary medicinal products or other treatments administered to the animals within a relevant period, and with a withdrawal period greater than zero

– the occurrence of diseases that may affect the safety of the meat

– the results, if relevant to the protection of public health, of any analysis carried out on samples taken from the animals

– relevant reports about previous ante- and post-mortem inspections of animals from the same holding of provenance

– production data, when these might indicate the presence of disease

– the name and address of the private veterinarian normally attending the holding of provenance.

For every individual shipment, the farmer, as a food-business operator, provides the required information to the abattoir. In addition, in Germany, private control systems, such as the 'QS Qualität und Sicherheit GmbH' (QS), can be used to audit procedures in primary production, ensuring that participants adhere to fixed standards (17).

Inspection

Traditional meat inspection follows Reg. (EC) No. 854/2004 (18). For pigs, this constitutes a visual examination of each surface and organ, palpation of some organs, and incision of the mandibular lymph nodes (Lnn. mandibulares), heart, trachea and lungs in carcasses destined for human consumption (18). If fit for consumption, carcasses are classified and chilled to 7°C, with offal being cooled to 3°C (5). Carcasses are then cut up and parts are further processed (tertiary production) or enter the retail trade as fresh meat.

Risk-based meat inspection

In risk-based meat inspection, the management infrastructure of the farm is regarded as being of special interest. Specific management and other requirements are listed in Reg. (EC) No. 1244/2007 (19). Some items are listed in Reg. (EC) No. 2075/2005 (20) for areas free of *Trichinella* spp. Regulation (EC) No. 854/2004 (18) allows risk-based meat inspection with 'visual only' examination for fattening pigs; this is established more precisely in Reg. (EC) No. 1244/2007 (19):

– pigs must be kept in controlled housing conditions in integrated production systems (Box 1)

– the competent authority implements or orders regular serological and/or microbiological monitoring of a selected number of animals based on a particular risk (specific items are not listed).

Information technology for data provision and processing

The identification of each farm and each animal is a legal requirement, undertaken by producers to conform with the law. An information technology (IT) infrastructure is required by national legislation (21), to collate data from the farm with the results of ante- and post-mortem inspections. Post-mortem information collected via inspection terminals is transferred to an IT centre where all other information comes together. Possible technical problems with terminal buttons and the system itself (22) can be prevented by internal verification of functioning (23). To prevent any loss of information, copies of all data should be made, with original data sets being kept at the place of generation.

For the transfer and allocation of results, a code scanner and a personal computer should be available. Each stakeholder (farm, abattoir, laboratory, Veterinary Service) should have access to the complete information pool.

Modern safety concerns cannot be completely addressed through traditional methods of meat inspection, which are directed more towards well-known animal diseases. They may be less useful in the work practices of today; in particular, because infected or contaminated animals generally do not show any symptoms (24, 25, 26). To overcome this problem, studying the food chain might provide more insight.

The aim of this study was to collect information from several stages of the food chain and from various disciplines, to gain insight into the particular circumstances of the chain and to fulfil food-chain requirements.

Materials and methods

Management parameters, meat-juice samples and post-mortem findings were collected from 296 indoor pig-fattening farms (conventional intensive production) in north-western Germany during the years 2005 to 2009.

Data collection

Data on a total of 56 management parameters were collected, mostly as yes/no information with no option for free text, from 296 farms in two farming associations (Association 1 contained 230 farms; Association 2 contained 66 farms) (Table I). Data from Association 1 were obtained during external QS audits; farmers in Association 2 were asked identical questions by telephone.

Commercial enzyme-linked immunosorbent assay (ELISA) test kits were used to test meat-juice samples for antibodies against salmonellae (SALMOTYPE® Pig Screen, LDL, Leipzig), *Trichinella* spp. (PrioCHECK Trichinella Ab®, Prionics, Zürich, CH) and *Yersinia* spp. (PIGTYPE® YOPSCREEN, LDL, Leipzig). Examination for salmonellae was carried out at an external laboratory, in accordance with mandatory German monitoring. The results were collected at the abattoir and transmitted to the Institute of Meat Hygiene and Technology laboratory. For antibodies against *Trichinella* spp. and *Yersinia* spp., samples were tested at the Institute.

Ten samples for *Trichinella* spp., six for *Yersinia* spp. and a maximum of ten for salmonellae were obtained from each farm, totalling 3,346 samples and 4,714 ELISA examinations.

Post-mortem findings were obtained from the veterinary meat inspection services at the abattoir. For this study, eight post-mortem parameters associated with hygiene were selected for further examination (Table II).

Methods

Information technology

Each farm was identified by an official registration number. At the abattoir, animals were tagged with a slaughter number and post-mortem findings were associated with individual numbers (via the abattoir terminal system for post-mortem inspection). Sample tubes were marked with a barcode and associated with the slaughter number of the individual carcass using a barcode scanner. Individual barcodes on all sample tubes were scanned before processing in the laboratory. Post-mortem findings were also associated with this number.

Parameters of farm management, ELISA results and post-mortem findings were entered into a total of eight tables under the registration number of the farm. Finally, two tables were generated with:

– management parameters plus laboratory data for each farm
– post-mortem findings for each year and individual farm.

The statistical programme PASW for Windows® was used for the analysis.

Step-by-step procedure for data analysis

Step 1:

Management parameters of the two farming associations (Table I) were compared (Association 1 versus Association 2).

Step 2:

All farms tested negative by ELISA for *Trichinella* spp.; thus only the results for salmonellae and *Yersinia* spp. were used in the analysis. Farms with a cut-off point lower than that set by the manufacturer were assumed to be negative, and farms harbouring animals with ELISA results higher than the cut-off point were classified as positive.

Farms that tested negative were compared with those that tested positive, with respect to their management parameters, as shown in Table I. Farms with negative results were also compared with the management decisions generally used in Association 2 (all negative farms were members of Association 2).

Step 3:

Post-mortem findings (Table II) of all farms (farm-related) were included in the analysis.

Step 4:

In the ELISA, the optical density (expressed as OD%) was frequently higher than the manufacturers' cut-off of 20 OD%. The threshold cut-off was therefore increased, which increased specificity and focused on farms with weaknesses in hygiene. The parameters were then defined as follows (see 'Serological testing', below):

– Salmonellae: cut-off 70 OD%, at least 3 of 10 samples > 70 OD%

– *Yersinia* spp.: cut-off 50 OD%, at least 4 of 6 samples > 50 OD%.

Farms with results beyond these limits were allocated the status 'highly burdened'. The management parameters of these farms were compared with those of negative farms again.

Step 5:

For post-mortem findings with an assumed hygiene association (Table II), farms were ranked separately for each parameter. Farms appearing more frequently at the top of these lists during the sampling period (2005 to 2009) were examined for individual management failures once again.

Results

Results of the step-by-step approach are shown in Figure 1.

Initial analysis of the farm management parameters from both farming associations found no significant differences, with the exception of 'transport only from this farm', 'feeding system' and 'management of deworming', which could be explained on the basis of the size of the farm and membership of one of the two associations.

All 296 farms tested negative for *Trichinella* spp.

Using the cut-off of 20 OD% (salmonellae and *Yersinia* spp.), 192 farms tested positive for both infections, 101 farms tested positive for one or the other, and three farms from Association 2 tested negative for all three infections. Comparing management parameters in the three negative farms did not yield any statistical evidence. A comparison of negative and positive farms identified 13 parameters, mostly associated with feed and feeding techniques, but the differences were not statistically significant.

An examination of post-mortem findings from all farms (Association 1 versus Association 2) also yielded no significant differences (the results are not shown here because the volume of the data set was nearly 145,000 data points per finding).

An increase in the cut-off points for salmonellae and *Yersinia* spp. drew attention to 22 farms that required closer examination. Post-mortem findings from these 'highly burdened' farms directed attention to three farms with high antibody titres against salmonellae and *Yersinia* spp., as well as suspicious post-mortem findings. Several management parameters found on these farms might have contributed to hygiene leakage and perhaps also to the spread of pathogens:

– the use of straw
– infrequent disinfection
– a continuous throughput of animals
– no drinking water other than a pulp-feeding system
– transportation to the abattoir by others.

Data quality: some farmers provided no information at all, which was considered suspect.

Discussion

Risk-based meat inspection, as described in Reg. (EC) No. 1244/2007 (19), was simulated in this study. Additional farm data were collected and meat-juice samples were examined for antibody testing against three zoonotic agents, among them salmonellae (mandatory in

Germany) and *Yersinia* spp. as a biosecurity indicator. *Trichinella* spp. serology was carried out, simulating the concept of *Trichinella* spp.-free farms (Reg. [EC] No. 2075/2005 [20]).

Serological testing

The antibody status of a herd is a useful tool for gaining insights into the epidemiology of infection in the herd and on the farm site; however, antibody status does not necessarily represent the present infection state of an individual animal (27). The use of serology alone can fail to detect newly infected animals (or farms) and ELISA testing may not be sufficiently comprehensive.

Nevertheless, ELISA testing permits the examination of large numbers of samples within a short period (28, 29, 30), and multiple serological examinations from a single sample are possible (31).

In this study, the manufacturers' cut-offs of 20 OD% for salmonellae and *Yersinia* spp. (high sensitivity, low specificity) were used. The results did not indicate any suspect farms. The cut-offs were therefore increased in 10% steps to increase specificity and to focus on farms with possible hygiene problems.

Risk status

Three pathogens were tested for: *Trichinella* spp. and salmonellae because of legal requirements (see 'Risk-based meat inspection', above) and *Yersinia* spp. because of their relevance to human health and biosecurity.

All pigs at the abattoir were examined for *Trichinella* spp. by digestion, with a negative result. Pigs on all farms tested negative for antibodies against *Trichinella* spp, an expected finding as the prevalence of these roundworms is known to be low in domestic pigs.

Antibody status for salmonellae and *Yersinia* spp. varied (negative for both, positive for both, or positive for one only). Farms with more than one pathogen were of interest: on those farms, problems with biosecurity or hygiene were considered to be more likely.

Comparisons of management parameters in the two farming associations and individual comparisons of positive and negative farms with respect to these parameters did not yield any significant differences.

After the increase in cut-off OD%, 22 farms were identified with animals demonstrating high antibody titres. In combination with frequent post-mortem findings, three farms with a list of suspect management parameters or which had problems with the quality of data provided were identified.

Bedding and infrequent disinfection

The use of straw and infrequent disinfection might contribute to the spread of *Ascaris suum* (32). On the other hand, straw is an important factor for animal well-being. Failures in cleaning and disinfection might increase the numbers of milk spots *post mortem*. In parallel, high numbers of milk spots reflect a lack of biosecurity measures, a finding that might also have consequences for the prevalence of salmonellae (33), as described for other agents (34). However, pigs infected with salmonellae, *Ascaris* spp. and additional parasites did not show any increase in salmonellae (35). In contrast, a correlation was found between milk spots and salmonellae in an abattoir-based study (33), possibly indicating that a high prevalence of *Ascaris* spp. is an indicator of inadequate biosecurity measures on a particular farm. On such holdings, salmonellae might find even easier ports of entry. It can be concluded that high levels of milk spots in correlation with the use of straw bedding and low disinfection rates indicate weaknesses in hygiene and biosecurity.

Continuous flow of animals

A continuous flow of animals facilitates the entry of zoonotic agents. Without effective cleaning and disinfection, the reduction or elimination of zoonotic agents cannot be achieved.

No additional drinking water other than via pulp-feeding

A lack of water might lead to animals licking pen mates or pen walls (36), which might promote the spread of zoonotic agents.

Transportation

In Association 2, animals were transported to the abattoir by outside companies, not in the farmers' own vehicles. The carry-over of pathogens in transporters that go from farm to farm is more likely than with being transported from one farm only (3), with the resulting controlled access at the animal site (Reg. [EC] No. 1244/2007). However, taking into account the management parameters in farms that tested negative and those in highly burdened farms, it was not possible to verify this conclusion.

Not all questions were answered

One farmer provided little information during the interview. This is basically a problem of data quality but can also be regarded as an additional pointer for hygiene weakness. An audit on the farm might circumvent the unwillingness of the farmer to answer questions during a telephone interview.

Data calculation

Data on management were obtained using yes/no answers in QS audits. However, the information was insufficiently precise to identify possible ports of pathogen entry or other hygiene weaknesses. A relationship between laboratory results and farm management practices was not instantly evident, which corroborates the findings of other studies (37, 38) in which the relation of management parameters to post-mortem findings was investigated. Moreover, QS audits take place for compliance with regulatory and industrial requirements and have not been developed for analytical purposes. Because several questionnaires for different purposes cannot be used in practice, the available set of questions from 'real' food-chain data sets was used, without additional analytical questions. Thus, yes/no answers may provide only the basic characteristics of a farm, as requested by

Reg. (EC) No. 854/2004 (18). More sophisticated information for risk-based meat inspection is required by Reg. (EC) No. 1244/2007 (19), and management parameters should be qualified (not only yes/no) in order to find a correlation between management factors and the antibody status of the herd.

In the present paper, the antibody status of individual animals was unknown; seroconversion against different agents should be kept in mind as a possible explanation for the lack of relationship. In addition, the impact of other factors, such as farm staff, the regional location of the farm, visitors, personnel movements on the site, or the presence of cats and dogs in the stables cannot be excluded and must be considered.

A step-by-step combination and specific examination of information provided the basis for detecting suspect farms. An increase in the ELISA cut-off OD% identified farms with a higher burden of infection and possible weaknesses in biosecurity. For these farms, management parameters were scrutinised further to detect circumstances possibly associated with a lack of hygiene measures or with the spread of pathogens. Local discussion at these sites might then improve farm biosecurity.

Conclusions

Different data sets reflect different items within a food chain, thus management parameters might indicate that all legal requirements are in place. For risk-based meat inspection, additional data are required, although the type and value of such information is still under discussion.

In this paper, basic QS audit information (farm management parameters) and indirect ELISA data were scrutinised. No obvious association was obtained, therefore additional data work was required.

A higher microbiological burden was identified by increasing the ELISA cut-off OD%, focusing attention on a small number of farms from which the available post-mortem data were analysed (parameters of hygiene relevance). Several management decisions indicating potential weak points were identified on three farms. It is concluded that each special case requires individual interpretation of its circumstances.

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Box 1

Requirements for controlled housing conditions and integrated production systems

(Appendix, Regulation [EC] 1244/2007)

- All feed has been obtained from a facility that produces feed in accordance with the requirements provided for in Articles 4 and 5 of Regulation (EC) No. 183/2005
- An all-in/all-out system is applied as far as possible; animals that are introduced into the herd are kept in isolation for as long as required by the Veterinary Services
- No access to outdoor facilities
- Detailed information is available concerning the animals from birth to slaughter and their management conditions (Section III of Annex II to Regulation [EC] No. 853/2004)
- If bedding is provided for the animals, the bedding material has to be treated to prevent diseases
- Holding staff comply with the general hygiene provisions (Annex I to Regulation [EC] No. 852/2004)
- Access of external persons is restricted to a minimum (no facilities for tourists or camping are provided)
- Animals do not have access to garbage dumps or household garbage
- A pest management and control plan is in place
- Silage feeding is not used
- Effluent and sediment from sewage treatment plants are not released into areas accessible to the animals

Table I

Farm management factors on which data were collected

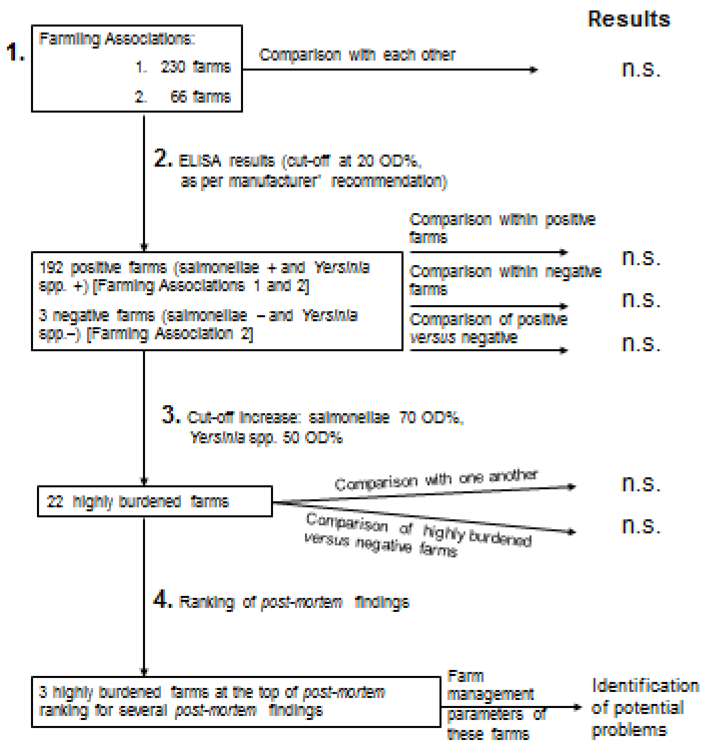
Animal contact	Animal health	Biosecurity	Buildings	Building surroundings
Contact with boar	Administration of therapeuticals	Pest control system	Pen (m^2/pig)	Surroundings of the sty
Contact with companion animals	Storage of medicine	Presence of flies (during audit)	Light management	Roads (structure)
Other pig holdings within 500 m	Management of deworming	Presence of pests (during audit)	Materials to enable pigs to perform normal activities of investigation and manipulation	
Poultry holdings within 500 m		Sty lockable	Quarantine pen (equipment)	
Cattle holdings within 500 m		Change or cleaning of boots	Floor	
		Disinfection of area in front of sty	Floor with straw	
		Epidemiological distance	Slatted floor (cm)	
		Plastic boots provided	Depth of slurry storage container (cm)	
		Protective clothes provided	Structure of building (during audit)	
		Fitting room exists		
Farm management	**Food and drink**	**Formal farm data**	**Hygiene management**	**Transport**
Fattening areas	Used food	Registration number	Cleaning	Transporter only for this farm
Number of stable units	Storage of food	Type of integrated system	Cleaning technique	Ramp exists
Pig multiplier	Cleaning of silos		Cleaning procedure	
Number of herds of provenance (piglets)	Feeding system		Disinfection	
Management of animals	Number of feeds per day		Disinfection technique	
Service period in days	Cleaning of liquid feeding system		Disinfection procedure	
	Source of water		Storage of cadavers	
	Drinking system		Cleaning and disinfection of the ramp	
	Filling of silo			

Table II
Post-mortem findings associated with hygiene

Post-mortem findings*	Possible aetiology	Reference numbers
Milk spots	Cleaning and disinfection; husbandry conditions	30, 39
Necrosis of the tail tip	Animal density	40, 41, 42
Necrosis of the nape of neck	Medical treatment; vaccination	21, 43
Bursitis	Husbandry conditions; animal density	44, 45
Inflammation of the intestine and its lymph nodes; swelling of the intestine wall	Infection	46
Suspicion of mycobacteria (lymph nodes of the intestine)	Port of entry at farm level	47
Abscess of the head	Infection** (also in correlation with intestinal infection)	48
Suspicion of *Mycobacterium* spp. (lymph nodes of the head); modification of the lymph nodes of the head	Entry; infection (also in correlation with intestinal infection)	46, 47, 49, 50

*Post-mortem findings were identified by inspection, palpation or incision, according to meat inspection techniques laid down in Reg. (EC) No. 854/2004 Annex I, Section IV, Chapter IV, No. A

**If an infection with *Mycobacterium* spp. is suspected (primarily *M. avium-intracellulare* complex), it is possible for lesions to occur in the lymph nodes of the pharynx, throat and intestine. These lesions cannot be differentiated by visual inspection alone from those of *M. bovis* (8) or others, such as *Rhodococcus equi* (51, 52).



n.s.: no significant results/differences

Fig. 1

Cross-connections of data (of variable quality and origin)