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Interruption of Microbial Cycles in Farm Animals from Farm to Table

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ABSTRACT

The fact that only slight problems are posed by Salmonella, Campylobacter and Escherichia coli O157 in mammalian farm animals in Norway, is undoubtedly due in large degree to the agricultural set-up with small farms and small herds, allowing a good overview of the situation at any time. Other factors are the very limited import of breeding animals and food products of animal origin, microbiological control of animal feed, and favourable geographical and climatic conditions. However, the Norwegian meat industry (including abattoirs) has continuing problems with Yersinia enterocolitica O:3 in pigs and pig meat, and in some cases with Listeria monocytogenes in cold cuts and Toxoplasma gondii in sheep meat (lamb). These three agents are therefore used to illustrate appropriate measures to be taken at the herd level on the farm, in abattoirs and in the meat processing industry. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

New and re-emerging infectious diseases have increased in humans during the past 20 years and have reached epidemic proportion in several countries. A substantial proportion of all re-emerging infections is associated with farm animals and meat. Among the agents involved are *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Escherichia coli* O157, *Listeria monocytogenes*, and *Toxoplasma gondii*. More intensive farming and production systems have contributed to the problem.

There are several points in the food chain from farm to table, at which control measures can be taken to prevent the spread of pathogens from mammalian slaughter animals via meat and meat products to man (Tables 1 and 2). The most relevant infectious agents are addressed, and preventive measures are specifically described for three agents of most importance under Norwegian conditions, namely *Y. enterocolitica*, *T. gondii* and *L. monocytogenes*. An insight is given into recent Norwegian research, in which analytical–epidemiological, serological, microbiological and genetic approaches have been used to identify measures to prevent the spread of these three agents to man as meat moves along the food chain from farm to table.

TABLE 1

Infectious agents of significance in the food production chain from farm to table, with an indication of where in the production chain, and with what effect, it is possible to introduce preventive measures

Agent	*Possibility for preventive action reduction in*				
	Herd/Flock	*Meat inspection*	*Slaughter*	*Cutting*	*Processing*
Salmonella spp.	+ + +	–	+ +	–	+
Campylobacter spp.	+?	–	+ +	–	+ +
Escherichia coli O157:H7	+?	–	+ +	–	+
Yersinia enterocolitica	+ + +	–	+ +	–	+
Listeria monocytogenes	–	–	–	–	+
Toxoplasma gondii	+	–	–	–	+

(+ + +, great effect; + +, good effect; + limited effect; –, probably of little effect).

TABLE 2

Main source of the agent

Agent	Main source
Salmonella spp.	Slaughter animals
Campylobacter spp.	Slaughter animals
Escherichia coli O157	Cattle
Yersinia enterocolitica	Pigs
Listeria monocytogenes	Environment
Toxoplasma gondii	Sheep

ORGANISMS OF LITTLE CONCERN

The fact that only slight problems are posed by *Salmonella, Campylobacter* and *E. coli* O157 in mammalian farm animals in Norway, is undoubtedly due in large measure to the agricultural set-up with small farms and small herds allowing a good overview of the situation at any time. Other factors are the very limited import of breeding animals and food products of animal origin, microbiological control of animal feed and favourable geographical and climatic conditions. In the marketing of Norwegian meat and meat products, one has used the slogan "small, cold, and remote" to represent this situation.

Salmonella spp.

Salmonella spp. are the most frequently reported bacterial agents of acute diarrhoeal illness in Norway. In 1994 the national surveillance system recorded approximately 1400 bacteriologically verified cases among 4.3 million Norwegians every year. During the past decade the number of reported cases due to *S. enteritidis* has increased considerably.

Salmonella bacteria in mammalian farm animals hardly pose any great problem for Norwegian slaughterhouses or the Norwegian meat industry. On the other hand, there is an endemic reservoir of *S. typhimurium* var. copenhagen in wild birds. This serovariant is also sporadically encountered in mesenteric lymph nodes from slaughtered pigs. A monitoring programme, similar to those implemented in Finland and Sweden, using

traditional microbiological diagnosis based on random sampling of 3,000 mesenteric lymph node samples from each animal species throughout the country showed that the incidence of *Salmonella* spp. in pigs and cattle was about 0.1% in 1995. This is an unique and favourable situation which we share with Finland and Sweden, in a global market with a considerable *Salmonella* problem. Moreover, epidemiological data from the National Institute of Public Health also show that mammalian slaughter animals are hardly likely to constitute a source of human salmonellosis in Norway today. About 80–90% of human cases in Norway are due to people being infected travelling abroad (G. Kapperud, personal communication).

Campylobacter spp.

Campylobacter jejuni/coli is the second most common bacterial agent of acute diarrhoeal illness in Norway. The number of culture-confirmed cases recorded by surveillance has increased considerably in recent years. While ca. 600 cases were reported in 1992, the number had risen to ca. 1,000 in 1994. About 50% of the patients had acquired the infection abroad.

In spite of a very high carrier rate of *C. coli* in slaughter pigs, it does not seem that consumption of meat from mammalian slaughter animals can be implicated in human cases of infection with this bacterium (Kapperud *et al.*, 1992). It appears that these bacteria are decimated in retail pork products, and more or less disappear during storage (Nesbakken *et al.*, 1985).

Escherichia coli O157

In an investigation organised by the Norwegian College of Veterinary Medicine, faeces from 1980 cattle from 198 herds were examined for *E. coli*. Two (1.0%) herds and six (0.03%) individual animals were found to be positive (Vold *et al.*, 1996). Although two human cases of infection with this bacterium were recorded some years ago, no cases have been reported in recent years. There is no epidemiological indication that the animal population represents any risk for the consumer in Norway.

ORGANISMS OF GREATER CONCERN

As mentioned above, the Norwegian meat industry (including abattoirs) has continuing problems with *Y. enterocolitica* O:3 in pigs and pork, and in some cases with *L. monocytogenes* in cold cuts and *T. gondii* in sheep meat (lamb). These three agents will therefore be used to illustrate appropriate measures to be taken at the herd level on the farm, in abattoirs and in the meat processing industry to reduce the health hazards implicated in the consumption of meat. The report presented here represents the results of cooperative projects between the Norwegian Meat Cooperative, Oslo, the National Institute of Public Health, Oslo, the Norwegian College of Veterinary Medicine, Oslo, the Danish Veterinary Laboratory, Copenhagen, and the Swedish Meat Research Institute, Kävlinge and of other published findings.

Yersinia enterocolitica

Approximately 200 cases of yersiniosis are reported annually in Norway (National Institute of Public Health, Oslo). The actual number of people becoming ill because of *Y. enterocolitica* is however at least 10 times higher than the number of reported cases

(Nesbakken, 1992). *Yersinia enterocolitica* is in Norway the third most common food-borne bacterial infection—surpassed only by *Salmonella* and *Campylobacter* infections—and almost all infections are caused by serovar O:3.

The pig and pork constitute the most important source of *Y. enterocolitica O:3* infection in man in Norway (Nesbakken, 1992; Ostroff *et al.*, 1994). The organism is often found in the intestinal contents and faeces of pigs. Shiozawa *et al.* (1991) found that 24.3% of 140 pigs were carriers of the organism in the caecum, counts varying from less than 300 up to 110,000 bacteria per gram of intestinal content.

At the farm level, new-born piglets are easily colonized and become long-term healthy carriers of *Y. enterocolitica* in the oral cavity and intestines (Schiemann, 1989). This phenomenon, together with the widespread occurrence of the bacterium at the herd level, has previously led to the suggestion that its control at this stage is hardly feasible (Nesbakken, 1992).

Occurence and relevant preventive measures at the herd level

In a recent study (Skjerve *et al.*, in preparation), we used an enzyme-linked immunosorbent assay (ELISA) to detect IgG antibodies against *Y. enterocolitica* O:3 in sera from 1630 slaughter pigs from 326 different herds, in order to ascertain if it was possible to identify herds with no prevalence of antibodies. Furthermore, we tried to identify sources of infection and guide control and prevention efforts at the herd level.

Blood samples were taken from pigs by employees at four of the largest pig slaughterhouses in Norway during the period November 1993 to October 1994. Five blood samples were taken from each of 326 randomly selected herds. Sera were analyzed using an ELISA method (Nielsen *et al.*, 1995). Positive herds were defined as herds with either one animal with a high titre, or with two or more seropositive animals out of five. All herd owners were mailed a standardized questionnaire with questions related to management, hygiene, house construction etc. Some days later, specially trained veterinary students contacted the farmers by phone and recorded the data on the same standard questionnaire.

Positive titres were found in 869 (53.3%) of the 1630 samples investigated, and 207 (63.5%) out of 326 herds were defined as "*Yersinia enterocolitica* O:3 herds". There were significantly fewer positive combined herds of piglets and fatteners than fattening herds. Moreover, one could ascertain that the purchase and movement of animals into these herds was a contributing factor to the positive carrier status. Relevant measures, as with *Salmonella*, will thus be to cull breeding sows which are carriers of *Y. enterocolitica* O:3. Possible follow-up measures in slaughterhouses and the meat processing industry to exploit the results achieved at the herd level could then be:

- adjustment of the price paid to the farmer (incentive bonus to carrier-free herds)
- meat originating from "negative" herds to be used as raw material in the form of fresh meat i.e. minced meat, fresh sausage meat, joints
- meat originating from "positive" herds to be used as raw material to produce heat-treated products.

Although such a two-way splitting of pig-meat production would pose a logistic problem, it should actually be possible in Norway, where one has a good overview of pig herds and identification of animals and marking of carcasses from slaughter to cutting, if the successful achievement of such a system were to be considered as a competitive advantage, and enough emphasis were to be placed on cost/benefit for public health.

Effective hygiene measures in slaughterhouses

It is not possible to sort out pigs contaminated with *Y. enterocolitica* at post-mortem meat inspection. Pig slaughter is an open process with many opportunities for the contamination of the pork carcass with the organism. However, it does not contain any point (Fig. 1) where hazards are completely eliminated (Borch *et al.*, 1996 in press). The major contamination points during pig slaughter are related to faecal and pharyngeal material (Andersen, 1988; Nesbakken, 1988). HACCP (Hazard Analysis Critical Control Point) and GMP (Good Manufacturing Practice) in pig slaughter must be focused on limiting this spread. The following assignment as critical points (CPs) or critical control points (CCPs) for specific steps during slaughter and dressing may serve as a guidance (Fig. 1): (i) lairage, (ii) killing, (iii) scalding, (iv) dehairing, (v) singeing/flaming, (vi) polishing, (vii) circumanal incision and removal of the intestines, (viii) excision of the tongue, pharynx, and in particular the tonsils, (ix) splitting, (x) post mortem meat inspection procedures, and (xi) deboning of the head.

In a further study (Nesbakken *et al.*, 1994), the practice of sealing off the rectum with a plastic bag during slaughter and dressing of pigs in one Norwegian and one Swedish

Process step	Hygienic aspect	Preventive actions	CP/CCP
Lairage	Contamination between animals	Cleaning & disinfection	CP
⇩			
Stunning			
⇩			
Killing	Contamination from tools	Cleaning & disinfection	CP
⇩			
Scalding	Reduction of bacterial levels Contamination of lungs (?)	Time/Temperature	CP
⇩			
Dehairing	Contamination from machines	Cleaning & disinfection	CP
⇩			
Singeing/flaming	Reduction of bacterial levels	Time/Temperature	CP
⇩			
Polishing	Contamination from machines	Cleaning & disinfection	CP
⇩			
Evisceration	Contamination from intestines Contamination from tongue, pharynx and tonsils Contamination from tools	Enclosure of rectum Working instructions Disinfection of tools	CCP
⇩			
Splitting	Contamination via splitter/saw	Line-speed Water temperature	CP
⇩			
Meat inspection	Contamination from inspection	Disinfection of tools	CCP
⇩			
Deboning of head	Contamination from head	Working instructions Disinfection of tools	CCP

Fig. 1. Hygienic aspects and preventive actions with respect to *Yersinia enterocolitica* during pig slaughter. CP, critical point; CCP, critical control point. Adapted from Borch *et al.*, 1996, In press.

TABLE 3
Slaughterhouses and slaughtering processes in Norway and Sweden

Slaughterhouse and procedures	*Norway*	*Sweden*
Category of slaughterhouse	EU*	EU*
Slaughter rate	90 pigs/hr	240 pigs/hr
Circumanal incision	Manual	Mechanical

*EU certified.

slaughterhouse (Table 3) was evaluated with regard to its effect on the spread of *Y. enterocolitica* to pig carcasses.

Pig carcasses were sampled on two days during slaughtering by swabbing an area of 50 cm^2. In all, 120 pigs were sampled in each country, half being slaughtered and dressed using normal procedures and half applying the plastic bag technique. Sites on the carcass which were likely to become contaminated with faeces were chosen. In addition, one site was chosen in order to register any contamination caused by the cleaving saw/splitter (Fig. 2). Examination for *Y. enterocolitica* was carried out using an abbreviated version of the Nordic Committee on Food Analysis (1987) method.

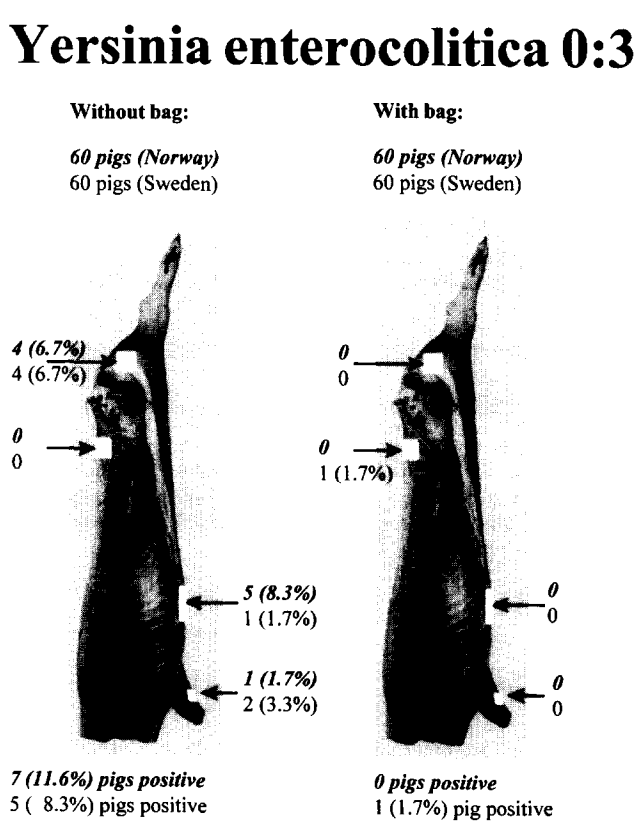

Fig. 2. Occurrence of *Yersinia enterocolitica* O:3

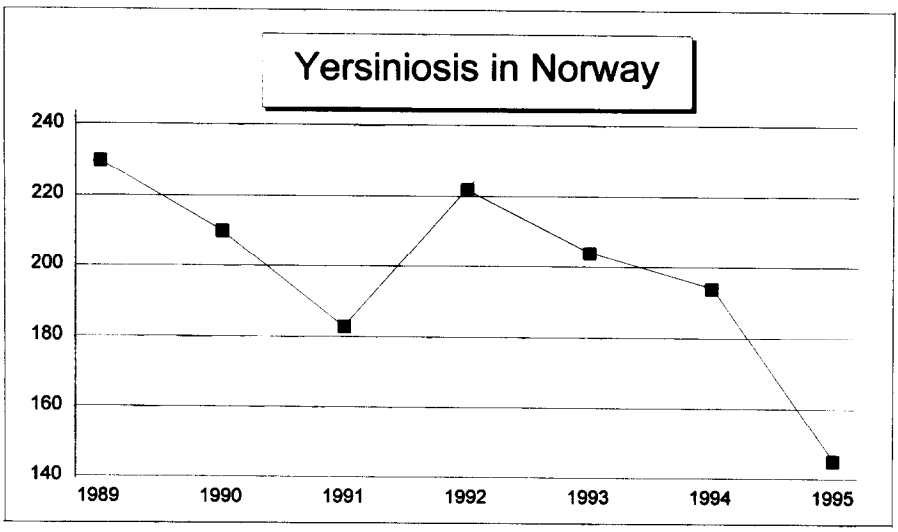

Fig. 3. Number of cases of yersiniosis in Norway. In 1994, the pig slaughterhouses in Norway started sealing-off the rectum with a plastic bag.

By sealing off the rectum with a plastic bag immediately after it had been freed, the spread of *Y. enterocolitica* O:3 to pig carcasses could be considerably reduced (Fig. 2). The organism was recovered from 10% of pig carcasses when eviscerating procedures did not include the use of the plastic bag technique (Fig. 2). There was thus an obvious risk of the bacteria further contaminating meat cuts and other meat products. The organism was recovered from only 0.8% of carcasses when the plastic bag technique was employed. The plastic bag technique was effective both in connection with manual excision of the rectum (low throughput ~90/hour), and mechanical freeing of the rectum (high slaughter rate ~240/hour) (Table 3). By incorporating the plastic bag technique into the slaughtering and dressing procedures, the meat industry would contribute to preventing the dissemination of *Y. enterocolitica* and other pathogens which spread via the faeces. The evisceration step might be a CCP (Fig. 1).

According to data from the National Institute of Public Health, yersiniosis has dropped about 25% after the introduction of the plastic bag technique in about 90% of the pig slaughterhouses in Norway (Fig. 3).

Toxoplasma gondii

The protozoan *Toxoplasma gondii* is one of the important zoonotic parasites common to man and most domestic animals. The importance of the parasite in reproductive failures in ewes (Hartley & Marshall, 1957) has long been recognized as a major problem in sheep husbandry. Up to 80% of abortions in ewes can be due to the parasite in many areas (Dubey & Beattie, 1988; Dubey & Kirkbridge, 1990; Dubey & Welcome, 1988). Since the organism was first described by Nicolle & Manceaux (1908), one of the main areas of interest has been its epidemiology in sheep. Although discussion has mainly focused on reproduction failures of ewes, attention has also been given to the risk of transmission of the agent to humans through consumption of raw or undercooked mutton. Approximately 500 million humans are today assumed to be infected with *T. gondii* (Dubey & Beattie, 1988).

Consumption of raw or undercooked mutton has recently been shown to constitute a major risk for pregnant women in Norway with regard to acquiring primary infection (Kapperud *et al.*, 1996 in press). If meat is frozen before sale/consumption, *T. gondii* will not represent a hazard, even though a few cysts might still be alive after weeks of freezing (Grossklaus & Baumgarten, 1968). An alternative would be the testing of sheep flocks before slaughter if the meat is to be sold as fresh meat. In any event, the consumer has to bear in mind the risk involved in eating any kind of meat without proper heating. Eating well-done cooked meat and keeping a high standard of hygiene in the kitchen will reduce the risk for acquiring not only *Toxoplasma* infections, but also a range of other possible meat-borne infections of parasitic and bacterial origin.

Occurrence and relevant preventive measures at the herd level

Norwegian sheep are raised under a wide variety of management systems, thus causing uncertainty as to the factors which are dominant in determining the risk of ewes or lambs acquiring the infection. As the life span of slaughter lambs only covers part of the year, the epidemiological determinants regarding infection in lambs may differ from those influencing abortion linked to infection during pregnancy. Lambs are usually slaughtered from the middle of September until November, while the mating of ewes takes place from late November until the middle of January.

The aim of a recent Norwegian study (Skjerve *et al.*, in preparation) was to update knowledge of the epidemiological pattern of *Toxoplasma* infection in slaughter lambs in Norway. More specifically, the aim was to identify epidemiological risk factors linked to management, grazing strategies, hygiene, and cat keeping, in order to formulate strategies to reduce the occurrence of *Toxoplasma* cysts in lamb meat.

The two largest slaughterhouses in each of the four most densely populated sheep districts were selected for sampling. All samples were taken during the autumn sheep slaughtering season, 1993. Blood samples were collected at bleeding from ten animals from each randomly selected flock.

A total of 207 randomly selected flocks were sampled during the study period. A positive flock was defined as a flock in which two or more of the animals tested were seropositive.

During the winter of 1994, all herd owners were mailed a standardized questionnaire on management, hygiene, house construction, grazing strategies, previous abortion problems etc. Some days later specially trained veterinary students contacted the farmers by phone and recorded the data on the same standard questionnaire.

Antibodies against *T. gondii* were detected in 44% of the 207 sheep flocks, while 17.8% of individual animals had antibodies. The main risk factors were linked to keeping a young cat daily in the sheep house, and atypical spring/autumn pasture near the home farm. The observed geographical differences were linked to altitude, with the higher incidences being found in areas >250 m above sea level. Farmers should be advised not to keep young cats in sheep houses, and to restrict near-farm grazing to a minimum. Considering, however, the high incidence of infection, the proposed preventive measures would not give the consumer sufficient protection and most of the lamb exposure seems to be from incidental contamination of cysts from cats. Ideally, fresh lamb meat should only be traded from herds tested for antibodies, or, alternatively, lamb meat should be frozen before consumption.

Listeria monocytogenes

Listeria monocytogenes causes listeriosis, characterised by symptoms of septicaemia and meningitis. Mortality is around 30% for certain groups at risk, and can even be as high

as 16% in non-risk groups (Goulet *et al.*, 1993). Annually, 3–21 cases are recorded in Norway (National Institute of Public Health, Oslo).

Although faeces and skin of slaughter animals are considered to be sources of *L. monocytogenes* contamination, the slaughterhouse environment has recently been more often implicated as an important source. As regards pigs, recent studies have shown a much higher incidence of the organism in the environment of the cutting room as well as on the primary cuts produced in this area, compared to the incidence at earlier stages of slaughtering (Van den Elzen & Snijders, 1993; Wendtland & Bergann, 1994). Often, only a few or no isolates are recovered from fresh pig carcasses (Nesbakken *et al.*, 1994). Results of an investigation using randomly amplified polymorphic DNA (RAPD) (Van den Elzen *et al.*, 1995) indicated that *L. monocytogenes* strains originating from pigs do not account for the contamination of the primary cuts. The significance of particular strains in the production environment has been emphasized by Boerlin & Piffaretti (1991). The use of multilocus enzyme electrophoresis (MEE) has shown that the natural population of *L. monocytogenes* is very diverse in terms of electrophoretic types (ETs). In spite of this genetic diversity, most human infections are caused by only a few ETs (Piffaretti *et al.*, 1989). MEE is a powerful tool for the study of the epidemiology of listeriosis, as it allows the distribution of *L. monocytogenes* clones in the environment to be assessed, and possible pathways of contamination of foods to be defined. Differences observed in the distribution of different clones could also reflect important differences between strains in their adaptation to diverse ecological niches, as well as differences in virulence among clones (Boerlin & Piffaretti, 1991).

MEE analysis of *L. monocytogenes* isolated from fish processing plants has shown that the ETs dominating in processed fish and in the processing environment, are different from those found in live fish and in sea water (Rørvik *et al.*, 1995). The investigation of Boerlin & Piffaretti (1991) indicated that this may also be the case with slaughter animals, fresh meat and meat products. Certain ETs among *L. monocytogenes* strains have often been isolated from meat, but not from animals. These findings indicate that contamination of meat with *L. monocytogenes* might originate mainly from the environment in which the meat is processed rather than from the animals themselves.

Pathways of dissemination of Listeria monocytogenes in meat processing plants

In a recent study (Nesbakken *et al.*, 1996 in press), MEE was used to characterize *L. monocytogenes* isolates from deboned fresh meat, the production environment, waste from slicers, and cold cuts and cured dried sausages, in order to trace the dissemination of the organism in meat processing plants. Isolates of the most frequently recovered ET (ET–6) were further differentiated by analysis of restriction fragment length polymorphism (RFLP) of the chromosomal DNA, using the enzyme *Hae* III.

One hundred and thirty-three isolates from deboned fresh meat, the production environment, and cold cuts, originating from five meat processing plants and from one plant producing cured dried sausages, were characterized using MEE. On the basis of electrophoretically demonstrable allelic variation at 21 enzyme loci, 21 ETs were distinguished. Analysis of the genetic relationships among the 21 ETs revealed two clusters: Cluster A and Cluster B. Most isolates causing human disease, both outbreaks and sporadic cases, belonged to clones of Cluster A.

With the exception of two isolates from one plant, all isolates from deboned fresh meat belonged to Cluster B. During processing of cold cuts, however, isolates belonging to Cluster A became more frequent, and only one of the 37 isolates from cold cuts belonged to Cluster B. In contrast, six of the nine isolates from cured dried sausages had ETs in Cluster B.

TABLE 4
Origin of *Listeria monocytogenes* on cold cuts

L. monocytogenes, *clusters**	*Slaughter animals*	*Deboned fresh meat*	*Processing*	*Cold cuts*
Cluster A	?	– (+)	+ + +	+ + +
Cluster B	?	+ + +	–	–

*Based on multilocus enzyme electroptoresis.
+ + +, majority of isolates.

One clone of Cluster ET–6 was isolated from cold cuts in four of six plants. This is one of the ETs most frequently recovered from patients in Norway and was responsible for a small outbreak (six cases) in the county of Trøndelag in 1992, and also caused a number of sporadic cases in recent years (D. A. Caugant, unpublished data). Using RFLP for analysis of chromosomal DNA six distinct restriction patterns were distinguished among the 44 ET–6 strains. In one plant, four different RFLP patterns could be identified. Two clone variants seem to have colonized different areas in this plant for at least four years. However, in each of the other plants, all ET–6 isolates had the same RFLP patterns.

In only one of the five plants producing cold cuts, was the same ET found both in the fresh meat and along the processing chain including the end-product. This indicates that the potential risk for contamination of the final end-product (cold cuts) by *L. monocytogenes* in the fresh meat, might have been overestimated. It is possible that personal and general hygiene in the packing room are more significant with regard to contamination of cold cuts. GMP and attention to CPs during processing in the meat plants are probably the most important prophylactic measures to avoid the presence of *L. monocytogenes* in cold cuts. A summary of the conclusions is presented in Table 4.

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