Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of domestic sheep and goats

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SCIENTIFIC REPORT OF EFSA

Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of domestic sheep and goats

European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

In this report, harmonised epidemiological indicators are proposed for food-borne biological hazards that are related to domestic sheep and goats and meat thereof and that can be addressed in the context of meat inspection. These hazards include *Toxoplasma gondii*, pathogenic verocytotoxin-producing *Escherichia coli* and mycobacteria. An epidemiological indicator is defined as the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates with the human health risk caused by the hazard. The indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be required, and to carry out risk analysis to support such decisions. It is foreseen that the indicators will be used in the integrated sheep and goats meat safety assurance system outlined in the EFSA Scientific Opinion on the public health hazards to be covered by inspection of meat from sheep and goats, particularly to help categorise farms/flock and slaughterhouses according to the risk related to the hazards and process hygiene. Depending on the purpose and the epidemiological situation, risk managers should decide on the most appropriate indicator(s) to use, either alone or in combination, at national, regional, slaughterhouse or farm/flock level. It is recommended that risk managers should define the harmonised requirements for controlled housing conditions of farms, and the requirements for food chain information. Member States are invited to report data generated by the implementation of the indicators in accordance with Directive 2003/99/EC. The proposed indicators should be regularly reviewed in light of new information and the data generated by their implementation.

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KEY WORDS


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2 Correspondence: zoonoses@efs.europa.eu
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SUMMARY

The European Commission requested that the European Food Safety Authority provides technical assistance on harmonised epidemiological criteria (harmonised epidemiological indicators, HEIs) for specific public health hazards in food and animals to be used by risk managers when they consider that the current methods of meat inspection do not adequately address the relevant risks. It is related to the mandate from the Commission for a Scientific Opinion on the public health hazards to be covered by inspection of meat.

In this report, harmonised epidemiological indicators are proposed for food-borne biological hazards that are related to sheep and goats and meat thereof and that can be addressed within meat inspection. These hazards include *Toxoplasma gondii* and pathogenic verocytotoxin-producing *Escherichia coli* (VTEC), as well as mycobacteria, since the latter are already covered by current meat inspection. An epidemiological indicator is understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain, or an indirect measure of the hazard (such as audits or evaluation of process hygiene) that correlates with a human health risk caused by the hazard. The epidemiological indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be required, and to enable Member States to carry out risk analysis to support any such decisions. It is foreseen that the epidemiological indicators will be used in the integrated meat safety assurance system for sheep and goats outlined in the Scientific Opinion on the public health hazards to be covered by inspection of meat from these animal species, particularly to help categorise farms/flocks and slaughterhouses according to the risks related to the main hazards identified.

The risk managers should decide on the most appropriate use of the epidemiological indicators at the European Union and national levels. Depending on the purpose and the epidemiological situation of the country, the indicators may be applied at national, regional, slaughterhouse or farm/herd level. The indicators can be used alone or in combination. They may be used to classify countries, regions or farms according to the infection status related to the hazards. Some indicators may also be used to evaluate the measures taken in the slaughterhouses to control a specific hazard.

The proposed indicators for *Toxoplasma* and pathogenic VTEC may be applied to classify the slaughter batches and animals according to the infection status or risks related to the hazard. For pathogenic VTEC, some indicators may also be used to evaluate the measures taken at the slaughterhouse to control the hazard or to assess process hygiene. In the case of *Mycobacterium*, epidemiological indicators are suggested to enable surveillance for the possible emergence of the hazard.

Most of the epidemiological indicators are proposed for subpopulations of sheep and goats at farm or slaughterhouse level using a variety of methods, such as visual inspection, serological or bacteriological tests. In the case of some of the biological hazards addressed it is accepted that there is a need for more research to clarify the factors that place sheep and goats at risk of infection, and the role of sheep and goat meat as a source of human infections.

The only comparable data available from the European Union Member States are those related to the official bovine tuberculosis free status that, in this report, has been proposed as one of the two harmonised epidemiological indicators for mycobacteria. No comparable data are available for the remaining proposed epidemiological indicators. For each epidemiological indicator addressed, the key elements of minimum monitoring or inspection requirements were defined. These included the animal population to be targeted, the stage of the food chain at which the sampling should take place, sampling strategy, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition.

At present, the majority of small ruminants are raised outdoors, and a considerable proportion are moved between premises during their lifetime. This excludes effective auditing of controlled
husbandry conditions as an indicator for a large proportion of the population. If these husbandry practices change towards more location stable and intensive practices, it is recommended that the possibility of applying an indicator on controlled husbandry conditions for additional hazards be reviewed.

The implementation of the proposed epidemiological indicators will generate additional data that will provide a more precise picture of the epidemiological situation in the European Union for these hazards, and these data may be used to update the indicators, when appropriate. It is recommended that the Member States report the data generated from monitoring these indicators in accordance with the framework prescribed in Directive 2003/99/EC. The proposed indicators should be reviewed regularly in light of new information and the data generated by their implementation. In addition, it is recommended that the potential indicators left out of this report owing to current data gaps and lack of evidence are reviewed and their inclusion considered if more evidence is obtained.
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BACKGROUND AS PROVIDED BY COMMISSION

Requests for technical assistance defining harmonised human health epidemiological criteria to carry out risk analysis within the scope of meat inspection

During their meeting on 6 November 2008, Chief Veterinary Officers (CVO) of the Member States agreed on conclusions on modernisation of sanitary inspection in slaughterhouses based on the recommendations issued during a seminar organised by the French Presidency from 7 to 11 July 2008. Inter alia, it was concluded that “EFSA and the European Centre for Disease Prevention and Control (ECDC) should define animal and human health epidemiological criteria required for the Member States to carry out their own risk analysis to be able, if appropriate, to adapt the general inspection methods within the framework provided by the legislation”. The CVO conclusions have been considered in the Commission Report on the experience gained from the application of the Hygiene Regulations, adopted on 28 July 2009. Council Conclusions on the Commission report were adopted on 20 November 2009 inviting the Commission to prepare concrete proposals allowing the effective implementation of modernised sanitary inspection in slaughterhouses while making full use of the principle of the 'risk-based approach'.

In accordance with Article 9(2) of Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EC and repealing Council Directive 92/117/EEC,⁴ EFSA shall examine and publish a summary report on the trends and sources of zoonoses, zoonotic agents and microbiological resistance in the European Union (EU) based on reports transmitted by the Member States. In addition, EFSA has prepared several scientific reports on (harmonised) monitoring of food-borne infections. Prevalence data from the zoonoses monitoring are considered as relevant epidemiological criteria to carry out a risk analysis, however, such data may be limited in certain Member States or not sufficiently harmonised to compare the situation between Member States. It is, therefore, appropriate to lay down harmonised human health epidemiological criteria and their minimum requirements. Such criteria should provide a tool to be used by risk managers in case they consider the current methods for meat inspection disproportionate to the risk.

In accordance with Article 20 of Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption,⁵ the Commission shall consult EFSA on certain matters falling within the scope of the Regulation whenever necessary.

TERMS OF REFERENCE AS PROVIDED BY COMMISSION

The scope of this mandate is to request technical assistance on harmonised epidemiological criteria for specific public health hazards in food and animals to be used by risk managers in case they consider the current methods for meat inspection address the relevant risk not adequate.


The following species or groups of species should be considered, taking into account the following order of priority identified in consultation of the Member States: domestic swine, poultry, bovine animals over six weeks old, bovine animals under six weeks old, domestic sheep and goats, farmed game and domestic solipeds.

In particular, EFSA is requested within the scope described above to:

1. Define harmonised epidemiological criteria for specific hazards already covered by current meat inspection (trichinellosis, tuberculosis, cysticercosis, …) and for possible additional hazards identified in a scientific opinion on the hazards to be covered by inspection of meat (see Annex 1), which can be used to consider adaptations of meat inspection methodology (e.g. prevalence, status of infection).

2. Provide a summary of comparable data from Member States based on the above defined harmonised epidemiological criteria, if existing, e.g. from ongoing monitoring in humans, food or animals.

3. Recommend methodologies and minimum monitoring/inspection requirements to provide comparable data on such harmonised epidemiological criteria, in particular if comparable data are missing. These criteria should also be achievable in small Member States.

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TECHNICAL SPECIFICATIONS

1. Introduction
There are a number of food-borne diseases affecting humans that can be related to consumption of sheep and goat meat and traced back to small ruminants. These hazards include parasites, bacteria and some viruses.

According to the Scientific Opinion of EFSA’s Panel on biological hazards (BIOHAZ), *Toxoplasma gondii* and pathogenic verocytotox in-producing *Escherichia coli* (VTEC) were considered of high public health relevance for sheep and goat meat inspection based on their high severity and on the evidence of meat from small ruminants as a risk factor for human disease (EFSA BIOHAZ Panel, 2013). Other meat-borne hazards of sheep and goats, such as *Bacillus anthracis*, *Campylobacter* and *Salmonella* were also identified in that Opinion, but their public health relevance was considered to be low, based on available data (EFSA BIOHAZ Panel, 2013). In addition, outbreaks linked to the consumption of meat from small ruminants have only very rarely been reported in the EU (EFSA and ECDC, 2012, 2013).

Meat inspection offers an opportunity to control some of the zoonotic hazards found in small ruminants. For example, mycobacteria are directly targeted through the current meat inspection procedures for small ruminants (Regulation (EC) No 854/2004). However, biological hazards that are currently found in small ruminants and considered of high public health relevance, as mentioned above, are not specifically addressed by the meat inspection system in place in the EU (EFSA BIOHAZ Panel, 2013).

It is possible to use the data on prevalence and concentration of the biological hazards in animals, meat and humans as one aspect of the criteria when determining and ranking the human health importance of the hazards to be covered by meat inspection. These epidemiological criteria or indicators may be used by risk managers when considering adaptations of current meat inspection methods for small ruminants. In the case of sheep and/or goats, data on the occurrence of zoonotic agents in animals and meat thereof are collected from the EU MSs within the framework of the annual reporting in accordance with Directive 2003/99/EC on the monitoring of zoonoses. Data on incidence of food-borne diseases in humans are collected by the European Centre for Disease Prevention and Control (ECDC) based on Decision 2119/98/EC on setting up a network for the epidemiological surveillance and control of communicable diseases in the EU.\(^\text{11}\)

The Scientific Opinion on the public health hazards to be covered by inspection of meat from sheep and goats (EFSA BIOHAZ Panel, 2013) proposes a new generic framework for small ruminant meat safety assurance. It is foreseen that the harmonised epidemiological indicators (HEI) will be used as part of this framework. Therefore, this report should be read in parallel with that Opinion.

2. Sheep and goat production in the EU
According to Eurostat, there were over 84 million sheep in the EU in 2011. The vast majority of them are found in Mediterranean countries and the United Kingdom. The United Kingdom and Spain are the leading sheep producers, accounting for over 45% of the EU’s sheep population (Figure 1).

\(^{10}\) In this report, the term small ruminant is used to refer to both domestic sheep and goats
Epidemiological indicators for meat inspection of domestic sheep and goats

Note: Data extracted on 4/10/2012 from the Eurostat statistics database (Eurostat, online). No data reported by Belgium, Denmark, Estonia, Ireland, Latvia or Finland.

Figure 1: Population of sheep in the EU, average of 2009, 2010 and 2011 (Eurostat, online).

There is considerable variation in sheep husbandry between MSs because farming practices have been developed to suit the culture, the land and the climate (Ashworth, 2000). Transhumance is an example, mainly from the Mediterranean countries, where sheep are grazed in the highlands in the summer and then relocated by shepherds to lowlands during the winter. This relocation can be several hundreds of kilometres. In Great Britain seasonal movement of sheep also occur from highlands to lowlands, but normally the responsibility for the animals is also temporarily transferred. Furthermore, stratification is practised in the United Kingdom, which generates a structured industry based on landscape type and adapted breeds (UK Agriculture, online). Generally, sturdy breeds are bred in the mountains and uplands and sold and moved to more favourable areas for rearing or further cross breeding.

In northern EU MSs sheep are mainly kept for meat production, producing heavy lambs, which have been weaned and reared (Ashworth, 2000). This is usually seasonal, with lambing in March-April and slaughter in August-September or over the winter. In the Mediterranean countries, sheep are often kept for milking, and lambs are sold as light lambs (< 25kg) at weaning. They either go directly for slaughter or are reared further. Lambing and slaughter are less seasonal in southern EU MSs than in the north. Husbandry is adapted to the climate; in some MSs, sheep are kept outdoors all year round, and in some countries they are housed during winter and lambing. Outdoors, they are mainly fed via grazing but, depending on the climate, additional feed may be provided during winter months and close to lambing. Milking sheep farming is slowly shifting from pastoral to more intensive system in southern EU MSs and even though the majority of holdings are likely to remain as small family businesses, a larger proportion of animals are likely to be kept in more intensive systems, some even with zero-grazing, in the future (Aggelopoulos et al., 2009; Mantecón et al., 2009).

Goat farming figures for 2011 in the EU reveal a population of circa 13 million heads according to Eurostat (compared for example with 84 million heads of sheep and 86 million heads of cattle for the same year). As with sheep, southern/Mediterranean countries (i.e. Greece, Spain, France, Italy and Portugal) plus Romania have traditionally been the main producers of goats in Europe. The Netherlands is the exception to this geographical pattern, where goat farming has become increasingly popular in recent years, jumping from 268 000 heads in 2002 to 392 000 heads in 2011. Together, these seven countries account for 90 % of the goat population in the EU (see Figure 2).
Note: Data extracted on 4/10/2012 from the Eurostat statistics database (Eurostat, online). No data reported by Belgium, Denmark, Estonia, Ireland, Latvia or Finland.

Figure 2: Population of goats in the EU, average of 2009, 2010 and 2011 (Eurostat, online).

Different goat production systems can be found across Europe, ranging from the more traditional extensive systems that breed goats for mixed meat and milk production, to intensive dairy farms exclusively dedicated to the production of milk for cheese making. In between these two there are semi-intensive/extensive farms, dedicated either to mixed meat and milk production or to just milk production. The extensive systems are characterised by being small, family-run holdings, which rely largely on grazing to feed the goats and with a low productivity. Intensive goat farms are on average bigger in size, with the animals housed year-round (therefore relying on feed such as silage, compound concentrate feed and hay or straw as a source of fibre), which use mechanised milking machinery, very much like modern cattle dairy farms. However, these farms represent a small proportion of all small ruminants in the EU. Semi-extensive systems are somewhere in between these two. There are significant geographical differences in terms of the type of farm output across the EU. According to Eurostat data for 2011, France, Spain and Greece are the main producers of goat milk. For meat, the top three producing countries are Greece, Spain and Romania. Also, according to the same data, the three countries with most holdings in the EU are Romania, Bulgaria and Greece, well ahead of the rest of the countries. Together with the national herd size data, this indicates that the average holding size in these countries is probably small, which would point to a less intensive rearing system in these countries than in, for example, France and the Netherlands, where more intensive goat dairy farms are common. In the southern EU MSs, goats are mainly kept for milking purposes and husbandry is similar to that of sheep. Husbandry is adapted to the climate; in some MSs, goats are kept outdoors all year round, and in some countries they are housed during winter and up to kidding. Outdoors, they are mainly fed via grazing but, depending on the climate, additional feed may be provided during winter months and close to kidding.

Information on the structure of the processing industry for meat from small ruminants has been summarised in the Scientific Opinion on meat inspection of small ruminants (EFSA BIOHAZ Panel, 2013). Interested readers are therefore directed to this document, which also contains graphs on meat production in the EU.
3. Definitions

For the purpose of this report, the following definitions will apply:

**Audit**: a systematic and independent examination to determine whether arrangements, activities and related results comply with the requirements set for controlled husbandry conditions, transport, lairage and slaughter methods and whether these arrangements and activities are implemented effectively and are suitable to achieve the desired objectives.

**Biosecurity**: implementation of measures that reduce the risk of introduction and spread of infectious (zoonotic) agents. It requires the adoption of a set of attitudes and behaviours by people to reduce risk in all activities involving domestic, farmed and wild animals and their products.

**Breeding animals**: males over one year of age and ewes or nanny goats that have given birth.

**Carcase**: the body of an animal after slaughter and dressing (Regulation (EC) No 853/2004).

**Controlled husbandry conditions**: a type of animal husbandry in which farmed animals are kept at all times and for their whole life under specific conditions, which effectively exclude all relevant risk factors or maintains a constant level of risk. Such conditions would be controlled by the food business operator with regard to feeding, hygiene and biosecurity of the holding and would be specific for each hazard.

**Ewe**: a female sheep of breeding age.

**Goat**: domestic animals of the subspecies *Capra aegagrus hircus*.

**Harmonised epidemiological indicator (HEI)**: prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazards (such as audits of farms or evaluation of process hygiene) that correlates to a human health risk caused by the hazard.

**Hogg**: a young sheep of either sex from about 9 to 18 months of age (until it cuts two teeth).

**Lamb**: a young sheep in its first year.

**Nanny**: sexually-mature female goat.

**Sheep**: domestic animals of the species *Ovis aries*.

**Risk factor**: a variable associated with an increased risk of disease or infection.

**Slaughterhouse**: establishment used for slaughtering and dressing animals, the meat of which is intended for human consumption (Regulation (EC) No 853/2004).
4. **Approach applied to select the epidemiological indicators**

4.1. **Harmonised epidemiological indicators**

In this report, the term ‘epidemiological indicator’ is used instead of ‘epidemiological criterion’ for the sake of clarity. A harmonised epidemiological indicator (HEI) is, in this context, understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain that correlates to a human health risk caused by the hazard. Indirect indicators of the hazards, such as audits of farms or transport, are also covered.

The purpose of the HEIs proposed in this report is to enable the European Commission (EC) and the Member States (MSs) to consider whether adaptations to meat inspection methods may be implemented at the MS level, and to enable the MSs to carry out a risk analysis (or components thereof) to support decisions on any such adaptations of meat inspection methods. For those hazards identified in the complementary Scientific Opinion (EFSA BIOHAZ Panel, 2013) as the most relevant in the context of meat inspection, the epidemiological indicators provide information to be used in the sheep/goat meat safety assurance framework proposed in the Opinion. This applies in particular to the process of classification of the farms/herds and slaughterhouses according to risk related to a particular hazard, as well as to the setting of related targets for final carcases. The indicators, either alone or in combination, may be used by risk managers at the national, regional, slaughterhouse or farm/herd level depending on the purpose.

The principles applied to the identification of the appropriate indicators in this reports are as follows:

- For each biological hazard, the prevalence of the agent at key points in the food chain, broken down by risk factors that may be used for risk-based sampling (e.g. type of production system, age of animals), is considered. The key points are those at which risk is first created, primarily on-farm, but also possibly points at which the hazard can enter the food chain (e.g. during transport and slaughter) and where the hazard reservoir occurs (e.g. wildlife).
- The key epidemiological indicator for a given hazard will almost always be the prevalence in the animal population or in the food.
- The identification of a range of risk factors is not, in itself, sufficient. The impact of these risk factors on public health must also be estimated when amendments to the current meat inspection methods are considered. The impact may be measured by estimating the prevalence of the agent in the populations subject to different levels of exposure to the risk factor.

In this report, the following approach is applied to select the HEIs (the first Terms of Reference (ToR)):

The hazard and, when appropriate, its life cycle is described. The current epidemiological situation within the EU, as regards both animals and humans, is evaluated and the role of meat from small ruminants as the source of human infection is discussed for each hazard.

- For each hazard, the main meat production chain related to small ruminants, and the risk and risk-reducing factors along the chain, as well as the meat inspection and other risk mitigation strategies are presented. This description includes an identification of possible epidemiological indicators.
- The possible epidemiological indicators are evaluated against selected criteria (i.e. their quality, appropriateness, data availability and feasibility) using a scoring system. The epidemiological indicators that received the highest scores are selected.
Following the selection of the HEIs, the available data from the annual reporting in accordance with the Directive 2003/99/EC are reviewed for comparable data from the MSs. The availability of comparable data is discussed in chapter 6 (the second ToR).

In the cases where no comparable data are available, harmonised monitoring requirements are proposed for each selected epidemiological indicator (the third ToR). These include the definition of the animal population to be targeted, the stage of the food chain at which the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition. A general description on how to choose the sampling strategy for each case has been presented in the EFSA’s scientific report on HEIs for swine meat inspection (EFSA, 2011).

4.2. The biological hazards addressed

The first ToR of the mandate for technical assistance from the Commission asks for the harmonised epidemiological indicators to be defined for specific hazards already covered by current meat inspection (such as trichinellosis, tuberculosis, cysticercosis, etc.). In the case of meat inspection of small ruminants these hazards are tuberculosis and brucellosis. However, as *Brucella* usually presents with unspecific clinical signs and is therefore not usually detected during meat inspection, this hazard was not addressed in this document.

In addition, according to the first ToR, the epidemiological indicators for possible additional hazards identified in the Scientific Opinion on the hazards to be covered by inspection of meat from sheep and goats (EFSA BIOHAZ Panel, 2013) should be addressed as well. The Scientific Opinion identifies *Toxoplasma gondii* and pathogenic VTEC as such hazards.
5. Epidemiological indicators for the biological hazards

5.1. Toxoplasma gondii

5.1.1. Biology and epidemiology

*Toxoplasma gondii* is an intracellular protozoan parasite with a worldwide distribution, and infection is common in warm-blooded animals and humans. The parasite was discovered more or less simultaneously in Tunisia and Brazil in 1908, and named after its crescent shape (toxo = bow, plasma = life) and the original host (the gundi) (Ferguson, 2009). *T. gondii* is the only member of the genus *Toxoplasma*, belonging to the phylum of *Apicomplexa*, together with other coccidian species, piroplasma and plasmodia. Despite the fact that there is only one genus of *Toxoplasma*, strain isolation in mice demonstrated variation in mouse-virulence, and early genetic studies demonstrated that mouse-virulent strains all belonged to a single clonal genotype. Typing more strains showed a highly structured population, with nearly all strains in Europe and North America belonging to one of three clonal lineages (types I, II and III) (Howe and Sibley, 1995). More recently, *T. gondii* from other continents, particularly South America, has been shown to be genetically more divergent, and high clonality and low diversity are considered to be associated with a high degree of human activity and a dominance of domesticated cats and intermediate hosts versus a sylvatic cycle (Ajzenberg et al., 2004). Currently, 15 separate haplogroups that define six major clades are recognised (with haplogroups 1, 2 and 3 corresponding to the original clonal lineages) (Su et al., 2012).

*T. gondii* has a heteroxenous life cycle, and nearly all warm-blooded animals can act as intermediate hosts, carrying tissue cysts of this parasite (Figure 3). However, sexual development of the parasite occurs only in domestic and wild cats, which are the definitive hosts. Definitive hosts shed millions of oocysts upon primary infection. Sporulated oocysts are very resistant and remain infective in moist soil or sand for up to 18 months (Frenkel and Dubey, 1973) and can be distributed mechanically, by vectors, wind, or rain, and can be transported to water by run-off.
The infection may be acquired by humans through the consumption of undercooked meat containing tissue cysts, through consumption of food or water contaminated with oocysts, or through accidental ingestion of oocysts when handling contaminated soil or cat litter trays. Human infections with *T. gondii* occur worldwide although the prevalence varies regionally. Foci of high seroprevalence (> 40 %) exist in Latin America, parts of Eastern/Central Europe, the Middle East, parts of South-East Asia, and Africa; relatively low seroprevalences (< 20 %) have been reported in North America, China and Scandinavia (Pappas et al., 2009). The acute phase of human infection is usually asymptomatic or may be accompanied by mild flu-like symptoms, such as lymphadenitis and fever. However, if a woman is infected for the first time during pregnancy, the parasite may cause a serious fetal infection, resulting in abortion or congenital lesions in the child’s brains, eyes or other organs. In addition, *T. gondii* infection persists in the form of tissue cysts, and can cause problems later in life. It is estimated that in the United Kingdom about two-thirds of cases of ocular toxoplasmosis are due to acquired rather than congenital infection (Gilbert and Stanford, 2000). If an infected host becomes immunocompromised (e.g. HIV infection, immunosuppressive treatment for organ transplantation, cancer therapy), reactivation of tissue cysts can lead to uncontrolled multiplication of parasites and potentially fatal disseminated toxoplasmosis. Toxoplasmosis outbreaks are rarely reported since most infected immunocompetent individuals show few or no symptoms after infection (Smith, 1993).

Sheep and goats are highly susceptible to *T. gondii* infection (Munday and Corbould, 1979; Buxton, 1998) and are generally exposed to cat-shed oocysts while grazing or from contaminated feed and...
bedding. In sheep, tissue cysts develop preferentially in heart and brain and are thought to persist for the lifetime of the host (Dubey, 2009). After experimental infection in goats, parasites can be detected in various tissues, and recently the use of quantitative polymerase chain reaction (PCR) demonstrated the concentration to be highest in lung and brain tissue (Jurankova et al., 2013). Similarly to women, ewes and nanny goats may infect offspring through congenital transmission of tachyzoites. *T. gondii* is an important cause of abortion in sheep and goats, resulting in substantial economic losses. In contrast to humans, it has been suggested that, in sheep, the parasite may be transmitted to subsequent pregnancies, as parasite DNA has been detected in various tissues in aborted lambs and in fetus-derived placental tissue or umbilical cord in live-born lambs (Duncanson et al., 2001; Williams et al., 2005; Morley et al., 2008; Hide et al., 2009). Nonetheless, horizontal transmission through ingestion of oocysts remains the most important route of infection in sheep (Innes et al., 2009a). A vaccine based on a S48 strain to protect against congenital toxoplasmosis in sheep and goats is available.

5.1.2. Current situation and trends in the EU

In 2011, 29 confirmed human cases of congenital toxoplasmosis (infants <12 months) were reported from 18 EU MSs, as described in Decision No 2119/98/EC, with a notification rate of 0.01 per 100 000 population. Levels for *T. gondii* seroprevalence among human populations may depend on regional origin and local consumer habits. In Europe, the seroprevalence of *T. gondii* infection in women of childbearing age regionally varies between 10 % (e.g. Norway) and more than 70 % (e.g. Germany) (Tenter et al., 2000). When comparing seroprevalence data for *T. gondii* it should be taken into account that the different serological methods used to obtain these data are not standardised (Tenter et al., 2000).

In 2011, 17 MSs provided information on *T. gondii* in animals (EFSA and ECDC, 2013). As in previous years, the highest proportions of *T. gondii*-positive samples (from serological examinations) were reported in sheep and goats (31.2 %), cats (24.3 %) and dogs (20.7 %), while 6.5 % of the tested pigs and 6.3 % of cattle were positive. The proportion of samples positive for *T. gondii* or antibodies to *T. gondii* in sheep and goats in 2011 (31.2 %) was higher than in 2010 (18.2 %), 2009 (24.4 %), and 2008 (23.9 %). The proportion of positive samples in all sheep and goats varied greatly among MSs. However, differences in proportion of positive samples may be explained by differences in detection methods, population parameters (especially age structure) and targeted sampling due to clinical disease or suspicion of an infection (abortion). Data on *T. gondii* are mostly derived from clinical investigations, although sampling in the context of national surveys, monitoring and surveillance has been also reported (EFSA and ECDC, 2013).

Elevated *T. gondii* seroprevalences of up to 65% in small ruminants are frequently reported in the Mediterranean region and in Greece (Tzanidakis et al., 2012). In a recent study conducted in 125 Scottish sheep flocks, the overall *T. gondii* seroprevalence at individual level was 56.6 % (Katzer et al., 2011). In a study in France conducted in 2007, the proportion of sheep carcasses carrying live parasites was 5.4 % (3–7.5 %) and molecular typing mostly revealed genotype II (Halos et al., 2010). In a recent survey in Norwegian dairy goats, the seroprevalence was 17 %, and 75 % of herds were found to have at least one seropositive animal (Stormoen et al., 2012). Although isolation of *T. gondii* from caprine tissues has been reported, no large-scale prevalence data are available on the presence of this parasite in goat meat products (Kijlstra and Jongert, 2008).

5.1.3. Meat from small ruminants as source of infection for humans

There are only a few toxoplasmosis outbreaks which have been attributed to the consumption of sheep and goat meat in the past, and raw or improperly heated lamb meat was considered as the most probable source of infection (Smith, 1993; Lake et al., 2002; Ginsbourger et al., 2012).

The relative importance and frequency of horizontal transmission via tissue cysts versus oocysts in a given population is unclear. In a European case control study, depending on the region, 30–63% of new infections in pregnant women were attributed to meat and 6–17% to soil (Cook et al., 2000). In
the USA, the proportion of human cases that are food-borne was also estimated to be around 50 % (Mead et al., 1999). However, a serological assay that specifically detects the immune response to sporozoites (the parasite-stage contained in oocysts) has recently been developed (Hill et al., 2011), and preliminary data show an unexpectedly high proportion (78%) of mothers of congenitally infected infants to be positive (Boyer et al., 2011) for sporozoite-specific antibodies. In other words, this finding would suggest that at most 22% of those mothers got infected through consumption of T. gondii-contaminated meat, indicating that oocyst-acquired infections may be more important than previously thought.

Considering meat-borne transmission, the risk of T. gondii infection from consumption of undercooked small ruminant meat is probably high in comparison with the risk associated with the consumption of pork, beef or poultry, as the prevalence of infection is higher in sheep and goats than in other food animals owing to the long persistence of tissue cysts in goat and sheep meat (Smith, 1993). However, the relative importance of small ruminant meat at a population level depends heavily on consumed amounts and preparation habits. Raw or undercooked lamb meat is a delicacy in certain countries such as France, where it is therefore considered an important source of T. gondii infection (AFSSA, 2005). Conversely, adult sheep meat is often well cooked and therefore probably poses a smaller risk of infection than lamb meat (Kijlstra and Jongert, 2008). A positive correlation between regional consumption of sheep meat and toxoplasmosis prevalence was demonstrated in a French study performed by Berger et al. (2007). Consumption of lamb was shown to be a strong risk factor for T. gondii infection (odds ratio (OR) 3.13; 95% confidence interval (CI): 1.4 - 7.2) in the European multicentre case control study carried out by Cook et al. (2000) with high population-attributable fractions in Oslo (21% of infected women), Brussels (10%) and Lausanne (10%). Salami and beef were more important than lamb in Naples, Milan and Copenhagen (Cook et al., 2000). In a quantitative risk assessment for meat-borne T. gondii infections in The Netherlands 14% of predicted infections were attributed to sheep (Opsteegh et al., 2011).

5.1.4. Risk and protective factors

Risks factors associated with T. gondii infection in small ruminants are age, presence of cats, grazing, source of drinking water, abortion history, absence of vaccination against T. gondii, geographical location and various other farm management characteristics.

Because, once infected, both the parasite and antibodies against T. gondii persist lifelong in small ruminants (and most other hosts), older animals, which have been exposed longer, are more likely to be infected. The prevalence of antibodies in ewes is generally more than twice that in lambs, and most sheep acquire infection before four years of age (Dubey, 2009). As the age effect is strong and robust, other risk factors can be evaluated reliably only in studies limited to a certain age group, or in multivariable, or at least age-adjusted, models.

Like herbivores, sheep and goats mainly become infected by ingestion of cat-shed oocysts. The presence of cats on farms was associated with an increased seroprevalence in sheep in Sicily (OR 2.8; 95% CI 1.7–4.5) (Vesco et al., 2007), as was the daily presence of a young cat in the sheep house in Norway (OR 4.11; 95% CI 1.01–19.7) (Skjerve et al., 1998). Cat numbers on farms should be limited, and because cats shed oocysts only upon primary infection, and cats that have the opportunity to hunt are generally infected at a young age, the presence of young cats in particular needs to be prevented. In addition, cats should be prevented from accessing feed and bedding material.

Grazing has been shown to increase the risk of oocyst uptake. In a Swedish study, the incidence of T. gondii infection in sheep was higher when sheep out on pasture than when housed indoors during winter, with seroconversion rates on pasture varying by location (Lunden et al., 1994). Variation by pasture location is also indicated in a Norwegian study in which grazing the lambs close to the farm (rather than sending them to a remote summer pasture) was identified as a risk factor (OR 6.35; 95% CI 2.36–17.11) (Skjerve et al., 1998).
The effect of grazing may be partly due to access to surface waters. *T. gondii* oocysts in the environment can be transported to water by run-off, and, experimentally, survival has been shown for 6 up to 54 months in water between 4 and 25 °C (Dubey, 1998). *T. gondii* oocysts have been detected in various types of water samples, including water well at farms (Sroka et al., 2006). *T. gondii* oocysts (10 by 12 μm in size (Dubey et al., 1998)) should be filtered out by most normally operating municipal water filtering systems that use coagulation, flocculation and settling prior to filtration (Jones and Dubey, 2010), indicating that tap water in most countries is safe. An increased risk of infection in sheep was shown when surface water rather than well water was used (OR 1.8; 95 % CI 1.1–3.1) (Vesco et al., 2007).

Some studies have identified other husbandry practices as risk factors, such as large flock size (Klun et al., 2006; Vesco et al., 2007), use of mouse poison and non-timber construction of the sheep house (Skjerve et al., 1998), grazing sheep together with sheep from other farms (Katzer et al., 2011), and cattle kept on the same premises (Hutchinson et al., 2011), but often with large uncertainty. No evidence is available to show whether eliminating these risk factors will have any impact on the presence or prevalence of *T. gondii* in the flocks or herds.

*T. gondii* is an important cause of abortion in sheep and goats, and abortion history has been associated with seroprevalence (Mainar et al., 1996). Nonetheless, abortion history as such is probably not a very informative factor when trying to classify farms as being of high or low risk of harbouring *T. gondii*. Firstly, a high abortion rate can be due to other causes and, secondly, a low abortion rate does not exclude a high *T. gondii* infection pressure, as, in that case, most sheep will be infected before pregnancy and are no longer at risk for *T. gondii*-induced abortion. Only a high abortion rate known to be caused by *T. gondii* indicates a high infection level in the flock.

A vaccine to protect against congenital toxoplasmosis in sheep is available in many European countries (e.g. United Kingdom, Ireland, France, The Netherlands, Spain, Italy), but, except for the United Kingdom, the vaccine is not widely used. Although the feasibility of vaccination to prevent tissue cyst formation has not been tested, vaccination limits parasite spread to the circulation of the host and thereby dissemination to the placenta as well as other tissues (Innes et al., 2009b). As vaccination is likely to reduce tissue cyst development, it can be considered a protective factor. However, because vaccination stimulates an immune response that currently cannot be differentiated from the response to natural infection, it can be associated with an increased seroprevalence.

Within Europe, geographical variation in *T. gondii* seroprevalence in sheep has been demonstrated in various countries, including Finland, France, Scotland, the Netherlands, Norway and Serbia (Skjerve et al., 1998; Klun et al., 2006; Halos et al., 2010; Jokelainen et al., 2010; Opsteegh et al., 2010a; Katzer et al., 2011). The regional differences can be due to climate-induced differences in oocyst survival but may also be due to regional differences in cat density or farm management factors that influence exposure to oocysts (e.g. cats on farms, grazing schedule and source of water).

Several factors influence the probability that a portion of small ruminant meat bought by the consumer contains infectious parasites. The concentration of tissue cysts is relatively low (<1 per 50 g, as suggested by Dubey et al. (1996)), and they are not homogeneously distributed (Esteban-Redondo and Innes, 1998). Therefore, larger portions and cuts from *T. gondii* predilection sites are more likely to be infected. In minced meat products, the meat of several animals is combined. Although this will dilute the concentration of parasites, the increased probability of contamination seems to increase the risk of human infection (Kapperud et al., 1996; Opsteegh et al., 2011).

Some products are treated before being sold to the consumer, for example by applying freezing or heat treatments. Freezing at less than –12 °C for at least two days or heating to a core temperature over 67 °C will render *T. gondii* non-viable (Dubey, 1996; Kijlstra and Jongert, 2008). Salting can also inactivate *T. gondii* depending on the salt concentration and duration (Kijlstra and Jongert, 2008).
Other treatments such as fermenting, drying and smoking reduce tissue cyst viability but the exact conditions needed are less well established (van Sprang, 1984; Kijlstra and Jongert, 2008).

Consumers may further reduce the risk of ingesting infectious tissue cysts by freezing meat products at home, or by heating them properly. Consumption of raw or undercooked lamb and mutton is an important risk for infection, which has been identified in several studies (Kapperud et al., 1996; Cook et al., 2000; Jones et al., 2009).

Although pregnant women and immunocompromised patients are at risk for a severe outcome, they are not more susceptible to infection, and their condition is not considered a risk factor for acquiring a *T. gondii* infection as such.

### 5.1.5. Proposed harmonised epidemiological indicators (HEIs)

In the previous section, risk and protective factors for *T. gondii* infection in small ruminants were identified and discussed. For these factors, potential epidemiological indicators were selected and assessed, as explained in section 4.1. The scheme describing these risk and risk-reducing factors in relation to the stage of the food chain and the evaluation of possible related epidemiological indicators is presented in Appendix A. Following the assessment, the most adequate HEIs for *T. gondii* in small ruminants were selected; these are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Harmonised epidemiological indicators for <em>T. gondii</em> in domestic sheep and goats</th>
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<td><strong>Indicators</strong></td>
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<td>HEI 1: Farms with controlled husbandry conditions</td>
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<td>HEI 2: Information on the age of the animals</td>
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<td>HEI 3: Detection of <em>T. gondii</em> infection</td>
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<td>HEI 4: Detection of <em>T. gondii</em> infection in older animals (more than one year) from farms with controlled husbandry conditions</td>
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<tr>
<td>HEI 5: Absence of <em>T. gondii</em> infection in younger animals (less than one year) from farms without controlled husbandry conditions</td>
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Auditing of farms for controlled husbandry conditions and categorising animals by age are applied in the proposed HEIs. Auditing was considered appropriate for *T. gondii* given the endemic nature of this hazard. Different analytical methods were assessed for the detection of *T. gondii* infection in small ruminants (see Appendix A), and finally serological testing of blood samples from small ruminants at the slaughterhouse was proposed. The correlation between the presence of antibodies and tissue cysts is assumed to be high in small ruminants (for more detailed information see Appendix B). As auditing of controlled husbandry conditions and age provide only limited information on their own, and the feasibility of serologically testing all animals is low (time-consuming and costly), we also propose to...
combine these indicators, limiting serological testing to categories based on age and husbandry conditions (Figure 4).

**Figure 4:** Schematic diagram illustrating the harmonised epidemiological indicators for *T. gondii* in domestic sheep and goats.

HEI 1 focuses on auditing the sheep and goat farms for controlled husbandry conditions, including control of cat access to the farm, feeding, water, etc. Proposed requirements for controlled husbandry conditions to reduce the risk of *T. gondii* in small ruminants are described in detail in Appendix C. Only intensive dairy sheep or goat farming is likely to meet all necessary requirements for controlled husbandry conditions. Although studies comparing the prevalence of *T. gondii* on these farms and conventional farms have not been reported, it is reasonable to assume that small ruminants raised under controlled husbandry conditions in the absence of cats are less likely to be infected with *T. gondii*.

HEI 2 focuses on the age of animals, and this information can be easily gathered from the food chain information system. As prevalence increases with age, older animals are considered of higher risk than young animals.

HEI 3 covers serological testing of blood samples from sheep and goats at the slaughterhouse, therefore providing an indication of the potential risk to public health. In addition, this indicator can also be used to gather data for surveillance purposes.

As mentioned above, serological testing of small ruminants (HEI 3) can be combined with HEI 1 and HEI 2, as proposed with the following indicators (HEI 4 and HEI 5).
HEI 4 focuses on animals older than one year raised under controlled husbandry conditions, which need to be tested at slaughterhouse for *T. gondii* antibodies, whereas younger animals are considered of low risk and may not be subjected to serological examination.

HEI 5 focuses on serological examination for *T. gondii* antibodies at slaughterhouse, of animals younger than one year raised under non-controlled husbandry conditions, which are considered as of low risk, whereas older sheep and goats are assumed to be of high risk, without the need for further testing.

Based on serological results (HEIs 3, 4 and 5), either individual animals (if all animals are tested) or slaughter batches can be categorised as low risk or high risk (EFSA, 2011). Animals with a negative serological result are classified as low risk (Figure 4), rather than negligible risk, because the presence of infectious parasites cannot be completely ruled out, as described in Appendix B. For that reason, it is also not possible to label any fresh unfrozen meat as *T. gondii* free.

Region was excluded as an HEI, because of the difficulties of disentangling true regional differences from regional variation in husbandry conditions. However, risk managers in countries with known areas with a low prevalence (e.g. the north of Finland) may want to consider all young animals in these areas as low risk without further testing, and also test older animals from conventional husbandry systems rather than systematically categorising all of them as high risk.

As the effect of vaccination on reduction of *T. gondii* tissue cyst development has not been well established, further research is needed before vaccination can be proposed as an HEI. However, in countries where vaccination of sheep is currently applied (e.g. the United Kingdom), serological testing is useful as an epidemiological indicator only for unvaccinated animals. In practice, vaccination is limited to breeding and replacement ewes from sheep farms without controlled husbandry conditions. Therefore, serological testing can still be applied to younger animals from these farms; however, the risk manager will need to decide whether to keep categorising vaccinated ewes as high risk without testing until the effects of vaccination have been demonstrated, or to categorise them as low risk assuming that vaccination prevents tissue cyst formation.

Risk categorisation of animals based on any of the proposed HEIs can be used to reduce the consumer’s risk of infection, e.g. by specifically using low-risk animals for high-risk products such as raw meat products and cuts that are more likely to be consumed undercooked, or by routing carcases of high-risk animals for heated or frozen meat products. Risk managers could apply this risk categorisation to individual animals (for example to assess the risk of toxoplasmosis derived from each carcase), or at batch level.

### 5.1.6. Harmonised monitoring requirements

#### Animal population

At the slaughterhouse:

- All domestic sheep and goats.
- Sheep and goats older than one year from farms with controlled husbandry conditions.
- Sheep and goats younger than one year from farms with non-controlled husbandry conditions.

Farms are subject to an audit for the controlled husbandry conditions.

#### Stage of the food chain

- The farm for audits of controlled husbandry conditions.
The slaughterhouse for sheep and goats older than one year from farms with controlled husbandry conditions or sheep and goats younger than one year from farms with non-controlled husbandry conditions or all sheep and goats at slaughter.

**Sampling**

**HEI 1**
- Target population: all sheep and goat farms claiming to meet the definition of controlled husbandry for *T. gondii*.
- Epidemiological unit: the farm.
- Sampling strategy: census (all farms claiming to meet the controlled husbandry conditions should be audited).
- Audit interval: repeated at a frequency (to be determined by risk managers) adequate to maintain confidence that farms continue to meet the definition of controlled husbandry.

**HEI 2**
- Target population: all animals.
- Epidemiological unit: individual animal at slaughter.
- Sampling strategy: census (information on age of all animals is available from food chain information).

**HEI 3**
- Target population: all domestic sheep and goats at slaughter.
- Epidemiological unit: depending on the risk management objective, individual animal or slaughter batch.
- Sampling strategy: all animals if carcases will be individually classified for risk management purposes. For batch classification, systematic or random sampling can be applied.
- Sample size: within batches it should be based on design prevalence (to be determined by risk managers) and group size, using methods for assessing freedom from *T. gondii* exposure (linked to a probability of freedom). For details of sample size determination and sampling, see Annex 3 of the EFSA report on swine meat inspection (EFSA, 2011).
  - If the indicator is intended only for surveillance purposes rather than risk management purposes, batches or carcases can be selected for sampling using probabilistic sampling methods, and stratified by subpopulation, e.g. slaughterhouses or region of origin, as relevant for the specific surveillance objective in the country.

**HEI 4**
- Target population: animals older than one year at slaughter, raised under controlled husbandry conditions.
- Epidemiological unit: depending on the risk management objective, slaughter batch or individual animals older than one year from controlled husbandry conditions.
- Sampling strategy: sampling for demonstration of ‘freedom from infections’ (linked to a probability of freedom) or census sampling from animals older than one year from controlled husbandry conditions (to be determined by risk managers). For details of sample size determination and sampling, see Annex 3 of the EFSA report on swine meat inspection (EFSA, 2011).
HEI 5

- Target population: sheep and goats younger than one year at slaughter raised under non-controlled husbandry conditions.
- Epidemiological unit: depending on the risk management objective, slaughter batch or individual animals younger than one year from non-controlled husbandry conditions.
- Sampling strategy: sampling for demonstration of ‘freedom from infections’ (linked to a probability of freedom) or census sampling from animals younger than one year from non-controlled husbandry conditions (to be determined by risk managers). For details of sample size determination and sampling, see Annex 3 of the EFSA report on swine meat inspection (EFSA, 2011).

Type and details of samples

- Blood samples are collected from sheep and goats at slaughtering. The blood is stored at room temperature to allow the blood to clot, then serum is separated and stored at −20 °C until the serological test. Pooling of samples should not be carried out.
- At the farm: questionnaire-based audit of farm procedures including specific conditions for T. gondii to be agreed by the risk managers (see Appendix C).

Diagnostic/analytical methods

- Detection of antibodies to T. gondii by blood serum test.
- Tests proposed are based on enzyme-linked immunosorbent assay (ELISA):
  - ELISA using formalin-fixed whole tachyzoites as antigen (Glor et al., 2013).
  - ELISA using SAG1 (P30) antigen: commercial kits use native antigen (Sager et al., 2003). Recombinant SAG1 antigen is also now available which can be used in a mixture of recombinant antigens (Velmurugan et al., 2008).

Note that the above tests are not officially validated at EU level.

Other tests are available, such as the modified agglutination test (MAT) or bead flow cytometry. MAT is limited in regard to large-scale testing of samples. Bead flow cytometry is still under development and is not yet ready for application under routine conditions.

Case definition

- The presence of T. gondii antibodies in a blood sample (individual animal level) results in classification as high-risk animal.
- The finding of a T. gondii-seropositive animal in a slaughter batch results in classification as high-risk slaughter batch.
- Animals older than one year are considered to pose a greater risk of harbouring T. gondii than younger animals.
- Farms found not complying with the controlled husbandry conditions (described in Appendix C) would be considered as posing a greater risk than farms with controlled husbandry conditions.
5.2. Pathogenic verocytotoxin-producing *Escherichia coli*

5.2.1. Biology and epidemiology

Verocytotoxin-producing *Escherichia coli* (VTEC) can cause serious illness in humans, with symptoms ranging from mild to bloody diarrhoea (haemorrhagic colitis), haemolytic–uraemic syndrome (HUS) and thrombocytopenia. VTEC is characterised by the production of potent cytotoxins, termed verocytotoxins, and comprises a genetically diverse group of *E. coli* strains, of which only a subset are considered to be pathogenic to humans. VTEC O157, O26, O103, O145, O111, O104 are the serogroups which have been most commonly linked to severe HUS illness in Europe, but illness has also been reported from a broad range of other VTEC serogroups. The pathogenicity of VTEC is related to the presence of the verocytotoxin gene in combination with other virulence-related genes but, according to a recent scientific Opinion of the EFSA’s BIOHAZ Panel (2013a), there is no single or combination of marker(s) that can now fully define a ‘pathogenic’ VTEC. However, in this Opinion it is concluded that any *E. coli* strains positive for the verocytotoxin gene (*vtx*) in combination with the *eae* ( intimin production) or *aaiC* (secreted protein of enteroaggregative *E. coli*) plus *aggR* (plasmid-encoded regulator) genes pose a risk of human VTEC infection. For the purpose of this report the term pathogenic VTEC is used to refer to those strains that cause disease in humans.

Small ruminants are reservoirs of a diverse range of VTEC serogroups and their potential as human pathogens can be assessed by screening isolates for the above combination of virulence genes. VTEC is not pathogenic in small ruminants and therefore no clinical signs can be observed. Animals can be exposed to VTEC via faecally contaminated grass, feed, water, other animals, environment, etc. Most information on VTEC colonisation of small ruminants relates to *E. coli* O157, and it is known that this serogroup can pass through the ruminant stomachs and colonise the terminal rectum. Colonised animals shed *E. coli* O157 for varying lengths of time and the numbers (load) of pathogen shed in the faeces also varies widely. Some animals, particularly those which are persistently colonised, can excrete exceptionally high numbers of *E. coli* O157 (> 10,000 colony-forming units (CFU)/g) in their faeces (Ogden et al., 2005; Franco et al., 2009). When colonised, small ruminants generally show no clinical symptoms of illness and re-infection occurs frequently, although in young unweaned lambs or kids scouring or diarrhoea may occur. Most knowledge about VTEC O157 in ruminants is derived from cattle, but it is likely that the bacteria–host relationship is similar in small ruminants. There is some evidence of higher shedding of O157 in adult sheep and hoggs than in lambs (Evans et al., 2011). The risk factors underpinning the different shedding patterns are poorly understood and knowledge in this area is also focused primarily on *E. coli* O157.

Transmission of VTEC from small ruminants to humans can occur by direct contact (hand to mouth) with contaminated faeces or indirectly via consumption of contaminated meat or contact with a contaminated environment such as water courses or soil or fresh produce grown or harvested in a contaminated setting. The relative importance of these transmission routes for human disease is unknown.

5.2.2. Current situation and trends in the EU

The epidemiological situation of VTEC in the EU is summarised in the EU Summary Reports on zoonoses and food-borne outbreaks (EFSA and ECDC, 2013).

The case classification of a confirmed human case of VTEC is defined in Decision (EC) No 2012/506/EU, and detection of VTEC is highly dependent on the methods applied to clinical

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specimens. Such methods vary markedly between different EU MSs, and VTEC O157 is more readily detected than non-O157 VTEC. Thus, data relating to non-O157 VTEC probably represent a substantive under-estimation of its true incidence, both for the EU as a whole and particularly for those MSs where molecular detection methods are not as yet fully utilised.

In 2011, a total of 9 485 confirmed human VTEC cases were reported by 23 MSs (EFSA and ECDC, 2013). This represents an increase of 159.4 % compared with 2010 (3 656 cases), mainly due to a large *E. coli* O104:H4 outbreak that occurred primarily in Germany, where it affected more than 3 816 persons, and with linked cases in an additional 15 countries. The overall EU notification rate of VTEC in 2011 was 1.93 cases per 100 000 population, more than double that in 2010 (0.83). There was a statistically significant increasing EU trend in the number of reported human cases of VTEC infection during 2008–2011. Even without 2011 data, and thus also excluding the VTEC O104:H4 outbreak cases, the EU increasing trend for VTEC infections during 2008–2010 remained significant (EFSA and ECDC, 2013). As in previous years, in 2011 the most commonly identified VTEC serogroup was O157, followed by the unusual O104 outbreak strain. Only two cases of serogroup O104 were reported in 2010 (EFSA and ECDC, 2013).

As with information on human cases, when interpreting the VTEC data from food and animals it is important to note that data from different investigations are not necessarily directly comparable owing to differences in sampling strategies and the analytical methods applied.

Twenty-two MSs reported data on VTEC in food in 2011 (EFSA and ECDC, 2013). Data were mostly reported on all VTEC or on the VTEC O157 serogroup. Only two MSs reported data on fresh sheep meat and carcases deriving from investigations with 25 or more samples, but all samples were negative for VTEC. One additional MS found that 14.5 % of the tested sheep meat samples at processing level were VTEC positive (*n* = 62). Between 2007 and 2010, eight MSs reported data on fresh sheep meat including 25 or more samples. Among those countries, only one MS reported VTEC-positive samples over that period. VTEC O157 was not detected in any fresh sheep meat sample in the last five years of investigations.

In 2011, 13 MSs provided data on VTEC in animals (EFSA and ECDC, 2013). Most of the reported data on VTEC were from cattle, in which VTEC was commonly found. Fewer data were reported in other animal species, and in only a few countries. Nevertheless, VTEC was found by two MSs from investigations on sheep. Only one MS reported data on goats, without any VTEC-positive finding. Between 2007 and 2010, three MSs consistently reported data on VTEC in sheep. Overall, no or low levels (< 2.0 %) of VTEC O157 were reported during that period, and the prevalence of VTEC varied widely, with levels ranging between 0 % and 77.7 %. In the years previous to 2011, data from goats were reported only in 2007 and 2010 by two MSs, with low or very low levels of VTEC.

Research studies on VTEC in sheep have focused mainly on O157, with reported prevalences of 7.1 % in adult sheep at slaughter in Italy (Franco et al., 2009) and 0.7 % in Great Britain in 2003, down from 1.7 % in 2000 (Paiba et al., 2002; Milnes et al., 2009). A Norwegian study found that 0.8 % of the national sheep flock carried VTEC O26 (Sekse et al., 2011) and a Spanish study found 8.7 % of sheep shedding VTEC O157 at slaughter (Oporto, 2008). Recent studies looked at an extended range of serogroups in sheep in Scotland (Evans et al., 2011) and Ireland (Thomas et al., 2013). The isolation rates from faecal material were 3.4 % for *E. coli* O157, 5.2 % for O26, 2.3 % for O103 and 0.1 % for O145 and on fleece samples were < 1 % for O157 and O26, with neither O103 nor O145 recovered from this matrix.

### 5.2.3. Meat from small ruminants as a source of infection for humans

There are some reports of lamb acting as a risk factor for human infection (Werber et al., 2007) and attributing consumption of sausages containing sheep meat to cases of VTEC infection in humans (Schimmer et al., 2008; Sekse et al., 2009). The evidence arising from epidemiological and source attribution studies points to a minor role for meat from small ruminants as a source of human cases of
VTEC, although the model used in the study carried out by Kosmider et al. (2010) was found to underestimate the observed prevalence of VTEC in lamb. Since small ruminants are considered to be an important source of VTEC strains that are virulent to humans, the potential risk to public health associated with VTEC cannot be ignored. Moreover, based on the high severity of disease and on the evidence of meat from small ruminants posing a risk for human disease, pathogenic VTEC has been considered as a hazard of high public health relevance for sheep and goat meat inspection (EFSA BIOHAZ Panel, 2013).

Meat from small ruminants becomes contaminated with VTEC in several ways. Firstly, the animal can be shedding VTEC, with the bacteria transferred directly from the fleece or intestines to the carcase during slaughter and dressing operation. Cross-contamination from other animals, carcases or the environment at slaughter, distribution, processing and consumer handling can also play a role in the risk of VTEC transmission to humans. Cross-contamination occurs frequently and the final VTEC status of a carcase is often independent of whether the animal was infected with VTEC when alive.

5.2.4. Risk and protective factors

The shedding of VTEC O157 in faeces is influenced by the age of the animals and is reportedly higher in older animals than in lambs or kids. Season also impacts on shedding, and in some MSs the prevalence of VTEC O157 in sheep peaks in the summer (Franco et al., 2009). Both Dutch and British studies have found a peak in late autumn/winter, and a Scottish study found a higher risk when cattle were housed indoors (Syngje et al., 2003; Ogden et al., 2004; Schouten et al., 2004) or failed to find any association with season (Milnes et al., 2008). Because the impact of season on the shedding of VTEC is not clear, this factor was not considered further.

Small ruminants are infected through a variety of pathways, since VTEC appears to be ubiquitous in farm environments. VTEC can be introduced by direct contact with faecal contamination from other animals including wildlife. It can also be introduced into a flock by feed, water, contaminated equipment and vehicles, or via introduction of new animals. Once present on a farm, VTEC survives well in the farm environment, including in water, bedding, feed and farm surfaces. Moist and warm environments particularly favour survival of VTEC, which enables colonisation of co-housed animals and thereby the maintenance of the VTEC burden in a flock. Measures to control the maintenance of VTEC on the farm include good hygiene to reduce the VTEC burden on farms and within flock, and maintaining stable rearing groups (Ellis-Iversen et al., 2008).

It is expected that, as reported for cattle (Gunn et al., 2007; Cernicchiaro et al., 2009), contact with other small ruminants from different flocks/herds by purchase of new stock, taking animals off the farm to visit agriculture shows or fairs and use of common grazing pasture all increase the risk of small ruminants being exposed to VTEC from other shedding animals.

Transmission of *E. coli* O157 and other VTEC can occur rapidly in groups of animals on farms, in transport and in lairage, with contamination of fleece taking place from faecally contaminated environments. Significant cross-contamination from animal to animal can occur during transport to the slaughterhouse and in the lairage. Mixing of animals from different farms and herds will increase the risk during transport and lairaging. The cleanliness and operation of transport vehicles and lairage arrangements influence the cleanliness and dryness of animals on arrival and in the pre-slaughter period. Small et al. (2002) showed that the most frequently contaminated sites in sheep lairages were unloading ramp floors, holding pen floors and water troughs. Pathogens have been reported to survive in the lairage area for more than one week, with survival rates being higher for bedding straw than for concrete or metal and higher for faecal contamination than for non-faecally contaminated areas (Small et al., 2003).

At slaughter, fleece and pelts represent a key source of microbial contamination of slaughter plants (EFSA, 2007b). Generally, visually clean animals produce less contaminated carcases than dirtier animals, although individual variation exists (Hauge et al., 2011). Dirty, dry fleeces are associated
with higher total viable counts, and the level of dirt also contributes to higher *Enterobacteriaceae* and coliform counts regardless of whether the fleece is wet or dry (Byrne et al., 2007). Other studies have shown no correlation between cleanliness of the animal coat and occurrence of pathogens including VTEC (McCleery et al., 2008; Thomas et al., 2012), although the methodology employed in these studies may have had an impact, as there are difficulties in swabbing and recovery of VTEC from heavily compacted, soiled animal coats.

To a lesser extent gut contents and faeces are a source of carcase contamination, but careful evisceration techniques with effective sealing of the oesophagus and rectum before removal of the stomach and intestines can reduce this risk. Personnel and equipment may also play a role in carcase contamination.

Operations which may reduce the number of VTEC organisms on carcases include trimming of visibly dirty areas of carcases, carcase washing (hot water at 74 °C (165 °F) for 5.5 seconds) (Bosilevac et al., 2006; EFSA Panel on Biological Hazards (BIOHAZ), 2010) and steam pasteurisation. The prevalence of pathogens on carcases is generally lower following chilling for 24 hours. However, the impact of chilling on the micro-flora is extremely variable because the industry does not refrigerate carcases in a uniform manner, with differences in temperature, air speed and relative humidity and resultant water activity of the carcase noted (Sheridan, 2004). There may also be methodological difficulties with recovery of bacteria from chilled carcases as the bacteria may be sublethally injured by the combination of chilling and reduced water activity, rendering them non-cultivable. During chilling some bacteria may become firmly attached to the meat or embedded into the meat issue and thus not readily recoverable by swabbing (Warringer et al., 2001).

5.2.5. **Proposed harmonised epidemiological indicators (HEIs)**

As with *T. gondii*, potential epidemiological indicators were selected and assessed. The scheme describing the food chain and related risk and risk reducing factors as well as the evaluation of possible epidemiological indicators are presented in Appendix A. The following epidemiological indicators have been selected for pathogenic VTEC in sheep and goats (Table 2, Figure 5).

**Table 2:** Harmonised epidemiological indicators for pathogenic VTEC in domestic sheep and goats

<table>
<thead>
<tr>
<th>Indicators (animal/food category/other)</th>
<th>Food chain stage</th>
<th>Analytical/diagnostic method</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEI 1: Occurrence of pathogenic VTEC in slaughter batch/group of animals one month before slaughter</td>
<td>Farm</td>
<td>Microbiology</td>
<td>Pooled faecal samples</td>
</tr>
<tr>
<td>HEI 2: Occurrence of pathogenic VTEC on fleece/pelt samples (after bleeding and before fleece/pelt removal)</td>
<td>Slaughterhouse</td>
<td>Microbiology</td>
<td>Fleece sample/pelt swab</td>
</tr>
<tr>
<td>HEI 3: Occurrence of pathogenic VTEC on carcases pre-chilling</td>
<td>Slaughterhouse</td>
<td>Microbiology</td>
<td>Carcase swabs</td>
</tr>
<tr>
<td>HEI 4: Occurrence of pathogenic VTEC on carcases post-chilling</td>
<td>Slaughterhouse</td>
<td>Microbiology</td>
<td>Carcase swabs</td>
</tr>
</tbody>
</table>
Figure 5: Schematic diagram illustrating the harmonised epidemiological indicators for pathogenic VTEC in domestic sheep and goats

There is a large data gap on the occurrence of pathogenic VTEC in the small ruminant chain at both farm and slaughterhouse level. Microbiological analyses at key points in the chain conducted by MSs using harmonised and standardised sampling and testing methodologies together with sero- and virulotyping of isolated VTEC will provide data on the occurrence of pathogenic VTEC (E. coli O157 and emerging serogroups) in small ruminants. Such data should in time allow for historical risk ranking of farms and for additional HEIs based on audits to be introduced at farm level.

HEI 1 classifies the slaughter batch of animals based on the occurrence of pathogenic VTEC in the slaughter batch or unit of animals one month before slaughter, using pooled faecal samples. Individual animals shed intermittently but this is rarely synchronised within a group of animals, and there will always be some animals shedding the bacteria, if VTEC is present. Interpreting results by aggregation at group level rather than individual level would increase the sensitivity of testing and the classification of the batch would be reliable. If samples are combined in pools, the sample sizes should be adjusted to account for the effect of pooling on test sensitivity. Sampling is recommended to be done one month prior to slaughter to assist risk management. Negative batches could be scheduled for slaughter on ‘clean days’ or directed to VTEC-negative slaughterhouses to avoid cross-contamination during the slaughter process. For surveillance purposes, routine testing will build up a harmonised picture of occurrence of VTEC at farm level.

HEI 2 provides information on the level of pathogenic VTEC present on the fleece/pelt as an indicator of the VTEC status of small ruminants entering the slaughter process. Because of the time delay in obtaining a result, this indicator will give data most relevant for surveillance purposes and, when linked to HEI 1, will also build up evidence of VTEC contamination occurring during transport and lairage.

HEI 3 provides an indicator of the occurrence of pathogenic VTEC on the small ruminant carcase pre-chilling. Sampling is performed prior to chilling rather than after chilling as it is easier to recover and cultivate VTEC bacteria at this point. Because of the time delay in obtaining a result, this indicator will
give data most relevant for surveillance purposes. Combining the results from HEI 2 and HEI 3 will allow the ability of the slaughter process to influence VTEC contamination of the carcases to be assessed. The low expected prevalence of VTEC on carcases will require large samples sizes to provide useful data.

HEI 4 focuses on providing an indicator of VTEC occurrence on carcases after the entire slaughter process (including chilling) has been completed. However, it is recognised that there are difficulties with sampling of chilled carcases as there is an active bacterial attachment to the carcase, making it difficult to recover VTEC via swabbing. Moreover, the bacteria may be stressed during chilling and in a viable but non-culturable state. The microbial levels found at this point in the process reflect the VTEC contamination level entering the food chain from the slaughterhouse. The data derived from monitoring of HEI 4 could be used to set VTEC targets for slaughterhouses. The low expected prevalence of VTEC on carcases will require large samples sizes to provide useful data.

The clean animal scoring system at entry to the slaughterhouse was excluded as an indicator, because of lack of evidence for association between VTEC occurrence and visually dirty fleece or pelts. This indicator could be included in the future if evidence for this association is derived.

The proposed HEIs give different types of information on the occurrence of VTEC infection in small ruminants and on contamination of the carcases and risk managers should choose the HEIs to be applied and then also interpret the available information in the appropriate way. The indicators may be used alone or in different combinations.

5.2.6. Harmonised monitoring requirements

Animal population

At farm:

- Slaughter batches or groups of animals (HEI 1).
  - Sampling of groups containing animals destined for slaughter to allow scheduled or directed slaughter. This will avoid cross-contamination during transport and slaughter processes of negative batches.

At slaughterhouse:

- Sheep and goats entering the slaughter line (after bleeding and before fleece/pelt removal) (HEI 2).
- Sheep and goat carcases pre-chilling (HEI 3).
- Sheep and goat carcases post chilling (HEI 4).

Stage of the food chain

- The farm (HEI 1).
- The slaughterhouse (HEIs 2, 3 and 4).

Sampling

HEI 1

- Target population: small ruminants destined for slaughter.
- Epidemiological unit: the group of animals that might be ready for slaughter one month later.
Epidemiological indicators for meat inspection of domestic sheep and goats

- Sampling strategy: representative sampling using a standardised methodology, e.g. by subdividing pens into smaller areas and using systematic or random strategies to select samples. For details, please refer to Annex 3 of the EFSA report on HEIs for swine meat inspection (EFSA, 2011).

- Sample size: within groups/batches should be based on design prevalence (to be determined by risk managers) and group size, using methods for assessing freedom from VTEC. For details, please refer to Annex 3 of the EFSA report on HEIs for swine meat inspection (EFSA, 2011).

- Survey interval: every consignment of animals destined for slaughter should be tested at each farm, a month prior to being transported to the abattoir.

**HEI 2**

- Target population: small ruminant animals after bleeding and before fleece/pelt removal.

- Epidemiological unit: depending on the risk management objective, individual carcases or at group level.

- Sampling strategy: representative sampling by random or systematic selection of carcases for testing. For details, please refer to Annex 3 of the EFSA report on HEIs for swine meat inspection (EFSA, 2011).

- Sample size: sufficient samples to confidently provide information relevant to the surveillance objective. To monitor prevalence and identify changes over time, guidelines for prevalence estimation should be followed as described in Annex 3 of the EFSA report on HEIs for swine meat inspection (EFSA, 2011). If the indicator is used to provide assurance of VTEC-‘free’ or low-risk slaughterhouses, the survey design would follow guidelines for demonstration of freedom and the unit of interest would be the slaughterhouse.

- Survey interval: repeated measurements at a frequency which provides the desired confidence and meaningful information for the surveillance objective.

**HEI 3**

- Target population: small ruminant carcases pre-chilling and after evisceration.

- Epidemiological unit: depending on the risk management objective, individual carcase or at group level.

- Sampling strategy: representative sampling by random or systematic selection of carcases for testing. Representative sample collected in accordance with Regulation (EC) No 2073/200513 (technique for pathogen sampling on carcases).

- Sample size: the sample size must be calculated to confidently provide information relevant to the surveillance objective. To monitor prevalence and identify changes over time, guidelines for prevalence estimation should be followed as described in Annex 3 of the EFSA report on HEIs for swine meat inspection (EFSA, 2011). If the indicator is used to provide assurance of VTEC-‘free’ or low-risk slaughterhouses, the survey design should follow guidelines for the demonstration of freedom and the unit of interest would be the slaughterhouse.

- Survey interval: repeated measurements at a frequency which provides the desired confidence and meaningful information for the surveillance objective.

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HEI 4

- Target population: small ruminant carcases after the slaughter process, and after chilling.
- Epidemiological unit: depending on the risk management objective, individual carcase or at group level.
- Sampling strategy: representative sampling by random or systematic selection of carcases for testing. Representative sample collected in accordance with Regulation (EC) No 2073/2005 (protocol for carcase sampling).
- Sample size: the sample size must be calculated to confidently provide information relevant to the surveillance objective. To monitor prevalence and identify changes over time, guidelines for prevalence estimation should be followed as described in Annex 3 of the EFSA report on HEIs for swine meat inspection (EFSA, 2011). If the indicator is used to provide assurance of VTEC-‘free’ or low-risk slaughterhouses, the survey design should follow guidelines for the demonstration of freedom and the unit of interest would be the slaughterhouse.
- Survey interval: repeated measurements at a frequency which provides the desired confidence and meaningful information for the surveillance objective.

**Type and details of sample**

- Pooled faecal samples either from animals or from the floor in the case of groups of animals ready for slaughter.
- Fleece sample (10 g) or pelt swab (site 400 cm$^2$) from the brisket area of the animal before fleece/pelt removal as outlined in the EFSA technical monitoring plan (2009).
- Representative sample collected in accordance with Regulation (EC) No 2073/2005 (protocol for carcase sampling).

**Diagnostic/analytical methods**


**Case definition**

- Finding of pathogenic VTEC in a sample, according to the recent Scientific Opinion on VTEC seropathotype and scientific criteria regarding pathogenicity assessment (EFSA Panel on Biological Hazards (BIOHAZ), 2013).
5.3. Mycobacteria

5.3.1. Biology and epidemiology

Tuberculosis is a serious disease of humans and animals caused by the bacterial species of the family Mycobacteriaceae, more specifically by species of the Mycobacterium tuberculosis complex (MTC). This group includes Mycobacterium bovis, responsible for bovine tuberculosis. This agent is also capable of infecting a wide range of warm-blooded mammals, including humans, goats and sheep. In humans, infection with M. bovis causes a disease that is very similar to infections with M. tuberculosis, the primary agent of human tuberculosis. Furthermore, the recently defined M. caprae also causes tuberculosis among animals, including sheep, goats and humans (Kubica et al., 2003; Rodriguez et al., 2009, 2011; Muñoz Mendoza et al., 2012).

The main transmission routes of these agents to humans are through contaminated food (especially unpasteurised milk and raw milk products from infected animals) or through direct contact with infected animals. Several wildlife species, such as deer, wild boar, badgers and the European bison, are important hosts for M. bovis and may contribute to the spread and/or maintenance of M. bovis infection in cattle in some MSs (EFSA and ECDC, 2013).

Infected sheep generally show few specific signs and may appear clinically healthy, even in advanced stages of disease. Tuberculous lesions in sheep show similar distribution and morphology to those observed in cattle, both grossly and histopathologically (Davidson et al., 1981; Malone et al., 2003; Marianelli et al., 2010). Acid-fast bacilli may be observed in the granulomata, but usually in very low numbers, and gross lesions need to be distinguished from those caused by Corynebacterium pseudotuberculosis (caseous lymphadenitis) (van der Burgt et al., 2013).

Goats also show few clinical signs of tuberculosis and the features of the disease are similar to those in cattle. Lesions associated with M. bovis and M. caprae are indistinguishable and are principally located in the respiratory tract (lungs and associated draining lymph nodes) (Crawshaw et al., 2008; Daniel et al., 2009; Quintas et al., 2010; Perez de Val et al., 2011). Acid-fast bacilli may be observed in the granulomata, but usually in very low numbers. Importantly, lesions may be found in the mammary tissues and mycobacteria isolated from the milk of infected animals.

Other mycobacteria occasionally produce disease clinically indistinguishable from tuberculosis. Mycobacterium avium complex (MAC) infection was recognised as the most common opportunistic bacterial infection in patients with acquired immunodeficiency syndrome (AIDS) (Cook, 2010). MAC includes eight mycobacteria species and several subspecies with different degrees of pathogenicity, a broad host range and environmental distribution in numerous biotopes including the soil, water, aerosols, etc. (Biet et al., 2005; Álvarez et al., 2011). Mycobacterium avium subsp. avium (MAA) is a potential zoonotic pathogen that belongs to MAC. Although several members of the MAC and other mycobacteria have been isolated from small ruminants, the most prevalent of these is Mycobacterium avium subsp. paratuberculosis (Map), which causes chronic enteritis in all ruminants. A link between this agent and the human chronic enteritis, Crohn’s disease, has been speculated and supported by several lines of evidence, such as the demonstration of Map-specific sequences in Crohn’s tissues. However, at present, there is no consensus on any aetiological role for Map in Crohn’s disease (Waddell et al., 2008; Chiodini et al., 2012; Wagner et al., 2013) and no evidence that it presents a risk via meat and meat products. Consequently, Map will not be considered further in this document. In humans, lymphadenitis due to non-tuberculous mycobacteria (NTM) primarily affects children and is caused by a variety of NTM, although M. avium predominates (van Ingen et al., 2010). In addition, other mycobacteria (e.g. M. kansasii, M. xenopi, M. malmoense, M. avium subsp. hominisuis) can cause NTM infections (Cook et al., 2010). NTM are not considered in this report, except the fact that all suspected lesions observed during visual meat inspection are sampled and sent to a diagnostic laboratory for subsequent investigation and characterisation. In addition, macroscopic lesions of NTM are often indistinguishable from true MTC lesions. For this reason, general rules for meat inspection for NTM should be applied in the same way as for M. bovis infection.
5.3.2. Current situation and trends in the EU

In 2011, 132 confirmed cases of human tuberculosis caused by \textit{M. bovis} were reported by 25 MSs (EFSA and ECDC, 2013), representing only 0.03 cases per 100 000 population. Most cases were reported in Germany, the United Kingdom and Spain, although the notification rate was highest in Ireland and The Netherlands (0.13 and 0.07 cases per 100 000 population, respectively). The number of confirmed cases of tuberculosis due to \textit{M. bovis} decreased in the EU in 2011 by 20.0 % after an increase in 2010 of 23.1 % compared with 2009.

In 2011, 11 MSs reported data on mycobacteria in small ruminants, of which only four MSs (having non-officially tuberculosis free (OTF) status in cattle) reported positive findings in sheep (0.03 %, \(n = 129\,085\)) and goats (1.2 %; \(n = 176\,926\)) (EFSA and ECDC, 2013). Two mycobacterial species were reported in 2011 in sheep and goats, \textit{M. bovis} and \textit{M. avium} subsp. \textit{paratuberculosis}. As in the previous years, \textit{M. bovis} was the most frequently reported mycobacterial species in small ruminants, although the vast majority of the positive samples from goats were reported as \textit{Mycobacterium} spp. A low prevalence in sheep and goats was also observed over the period 2004–2010; when overall only seven MSs reported sheep and goats positive for mycobacteria (i.e. \textit{M. bovis}, \textit{M. caprae} and unspecified mycobacteria) (EFSA, 2006, 2007a, 2009b, 2010; EFSA and ECDC, 2011, 2012, 2013).

Although \textit{M. bovis} is the predominant mycobacterial species reported from sheep and goats across the EU, in Spain \textit{M. caprae} now represents 7.4 % of all \textit{M. tuberculosis} complex isolates from domestic and wild animals and more \textit{M. caprae} infections in cattle were observed in regions with a high goat density (Rodriguez et al., 2011). This agent therefore may be of increasing concern.

During the years 2006–2011, the proportion of existing cattle herds infected or positive for \textit{M. bovis} in the EU was relatively stable at a very low level and ranging from 0.37 % in 2007 to 0.60 % in 2011. In addition, several MSs have reported the presence of \textit{M. bovis} and other mycobacterial spp. in wildlife.

5.3.3. Meat from small ruminant as a source of infection for humans

The genus \textit{Mycobacterium} includes several species that cause tuberculous infections in humans and other animals. Even though subclinical infections are more common than clinical infections, sheep and goat meat are unlikely to be a source of exposure for humans, based on the infrequent reporting of tuberculosis cases in these animals (even in MSs or zones where bovine tuberculosis is endemic), the absence of bacteria in blood of goats with lesions (Perez de Val et al., 2011) and the lack of documented evidence that sheep and goat meat is associated with human infection (EFSA BIOHAZ Panel, 2013). Furthermore, carcasses containing signs of tuberculosis infection are completely or partially condemned during routine meat inspection and any bacteria that might still be present in or on the meat would be killed by normal cooking. Human infection occurs mainly via other foods (e.g. raw milk) or occupation (e.g. direct contact with infected animals) or via the animal environment (inhalation).

5.3.4. Risk and protective factors

Sheep and goats are susceptible to several mycobacterial infections, including \textit{M. bovis} and in goats particularly \textit{M. caprae}, which can be acquired from imported animals, wildlife or the environment, depending on the agent. Badgers, deer and wild boar were considered to be the wildlife species posing the greatest potential risk of transmitting \textit{M. bovis} to cattle and other domesticated livestock in Europe (Naranjo et al., 2008; Wilson et al., 2009). \textit{M. caprae} has been reported in several wildlife species, but their role as reservoirs and modes of interspecies transmission has not been investigated extensively (Rodriguez et al., 2011).

Tuberculosis in sheep is rare, and sheep is regarded as a spill-over or amplifier host for \textit{M. bovis} (Coleman and Cooke, 2001) as cases are usually reported from regions where \textit{M. bovis} is endemic or involve contact with infected cattle. Considering these features, the main risk factors are purchase of or contact with infected animals and wildlife, particularly in regions/MSs that are not OTF. Currently,
the main risk-reducing factor at the farm level consists of applying correct biosecurity measures (e.g. use of fences). Domestic goats also appear to act as a spill-over host for *M. bovis* and the same risk-reducing measures can be implemented. An additional risk-reducing factor occurs at the slaughterhouse by the implementation of effective meat inspection to detect suspect (granulomatous) lesions. Because other pathogens can induce grossly similar lesions, it is important that suspect lesions are sampled for further laboratory investigations to confirm the presence of pathogenic mycobacteria and identify the species.

However, in contrast to sheep, goats are a maintenance host for *M. caprae*. Infected goats are considered to be the main reservoir for *M. caprae*, posing a risk for other goats, particularly those on the same premises, other domestic livestock and wildlife and humans (Rodriguez et al., 2011). Currently, the main risk-reducing factor consists of implementing controlled conditions at the farm, including correct biosecurity measures (e.g. use of fences, replacement purchase). A crucial adjunct to these measures is the effective pre-slaughter detection of infection in live goats on the farm, and several immunological assays, including an assay to detect gamma interferon (IFN-γ), have been reported for pre-slaughter detection of *M. bovis* and *M. caprae* infection in live animals (Bezos et al., 2011, 2012a; Shuralev et al., 2012). These tests show an adequate specificity in most situations and some MSs apply control programmes based on these tests. However, certain factors such as *C. pseudotuberculosis* infection and paratuberculosis vaccination can interfere with the interpretation of test results (Bezos et al., 2012b; Chartier et al., 2012). At the slaughterhouse, the same meat inspection procedures used for detection of *M. bovis* in sheep and goats are appropriate.

### 5.3.5. Proposed harmonised epidemiological indicators (HEIs)

Factors that could potentially be used as epidemiological indicators were selected and assessed. The scheme describing the food chain and related risk and risk reducing factors as well as the evaluation of possible epidemiological indicators are presented in Appendix A. As a result of this assessment, the following epidemiological indicators have been selected for mycobacteria in sheep and goats (Table 3, Figure 6).

**Table 3:** Harmonised epidemiological indicators for mycobacteria in domestic sheep and goats

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Food chain stage</th>
<th>Analytical/diagnostic method</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEI 1: Official bovine tuberculosis status&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Farm/region/Member State</td>
<td>Official records, food chain information</td>
<td>Not applicable</td>
</tr>
<tr>
<td>HEI 2: Human-pathogenic mycobacteria in sheep and/or goats at slaughter</td>
<td>Slaughterhouse</td>
<td>Visual meat inspection and microbiology&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>Suspected lesions</td>
</tr>
</tbody>
</table>

<sup>(a)</sup> Only for *M. bovis* in cattle.

<sup>(b)</sup> Detection and identification of the agent from lesions detected through visual inspection (culture and/or PCR).
HEI 1 takes advantage of official data for bovine tuberculosis that establish the OTF status of a MS, region or even farm. In conjunction with the fact that sheep and goats are a spill-over host for \textit{M. bovis} in MSs/regions where bovine tuberculosis is endemic and that the prevalence of pathogenic mycobacteria in small ruminants is very low, this indicator supports assessment of higher and lower risk for bovine tuberculosis in sheep and goats prior to slaughter. The information on the OTF status could also be included in the food chain information.

HEI 2 focuses on inspection of all slaughtered animals at the slaughterhouse. This HEI is based on visual inspection of sheep and goat carcases at slaughter and confirmation of the presence and species of pathogenic mycobacteria in suspicious lesions by microbiological testing. Because the prevalence of pathogenic mycobacteria in sheep and goats is very low, this indicator is more suited for monitoring and surveillance for detection of emergence of mycobacterial infections in sheep and goats populations. It also contributes to evidence of freedom from disease. However, the current situation may change in goats with respect to \textit{M. caprae}, which has been observed more frequently in recent years.

Considering some limitations of the serological testing, such as lack of sensitivity, specificity and the poor detection of more advanced clinical cases, serological testing was not proposed as analytical method.

Controlled husbandry conditions were considered as a potential indicator for sheep. However, it was concluded that it would not be an appropriate indicator as it is strongly influenced by the bovine OTF status of the farm, region or country (e.g. sheep farms with poor biosecurity would not necessarily have a higher risk of \textit{M. bovis} if they were in an OTF region or country).

For goats, controlled husbandry conditions and mycobacteria infection in live goats, were initially also considered as potential indicators. These were not finally retained in Table 3 because they primarily relate to infection by \textit{M. caprae}, which has not yet been demonstrated to be endemic in most MSs. These potential indicators provide information on the health status of goats at the farm but not on the...
human health risk. An indicator targeting the controlled husbandry conditions at the farm for goats, including biosecurity, could be used in the future to indicate whether adequate measures (infrastructure, management) are in place to minimise and avoid transmission between farms/regions and the introduction of infection to free farms/regions, particularly concerning \textit{M. caprae}. The robustness of these measures could be assessed by auditing farm records, supplemented by on-farm inspection, where required, to provide an indication of higher/lower risk for pathogenic mycobacteria prior to slaughter. An additional indicator could be used in future to specifically target \textit{M. caprae} infection in live goats at the farm. This farm-level indicator could rely on assays to assess specific cellular immune responses, such as the Single Intradermal Comparative Cervical Tuberculin (SICCT) test and IFN-γ ELISA (Bezos et al., 2012). Although the IFN-γ ELISA appears to be useful for this purpose in goat herds, only limited data are currently available (Bezos et al., 2012a).

5.3.6. Harmonised monitoring requirements

\textbf{Animal population}

- Cattle populations in MS/region/farm to establish OTF status (official records).
- All sheep and goats at the slaughterhouse.

\textbf{Stage of the food chain}

- The slaughterhouse.

\textbf{Sampling}

\textbf{HEI 1}

- Target population: cattle to establish the bovine OTF status of the MS, region or farm.
- Epidemiological unit: mainly MS or region, but can also apply to individual farm.
- Sampling strategy: as defined in Council Directive 64/432/EEC\textsuperscript{14} and prescribed by risk managers.
- Audit interval: as prescribed by risk managers.

\textbf{HEI 2}

- Target population: all sheep and goat carcases at the slaughterhouse.
- Epidemiological unit: the carcase.
- Sampling strategy: visual inspection to detect suspect lesions for further laboratory investigation. This would confirm the presence and species of pathogenic mycobacteria by microbiological testing.
- Audit interval: ongoing as all animals are inspected.

\textbf{Type and details of samples}

- All suspect lesions observed during the visual meat inspection are sampled and sent to a diagnostic laboratory for subsequent investigation using bacterial culture and molecular identification of pathogenic mycobacteria.

\textbf{Diagnostic/analytical methods}

- Official data on the bovine OTF status of the MS, region or farm.

Pathogenic mycobacteria detected in suspect lesions by culture (most common) or PCR and the species confirmed by molecular procedures (PCR, sequencing).

**Case definition**
- OTF MS/region/farm as defined in Council Directive 64/432/EEC.
- Detection of pathogenic mycobacteria in samples at slaughter.

**6. Comparable data on the harmonised epidemiological indicators**

The only comparable data available from the EU MSs are those related to the official bovine tuberculosis status that, in this report, has been proposed as one of the two harmonised epidemiological indicators for mycobacteria (see section 5.3.5). Data on bovine tuberculosis used to establish the official bovine tuberculosis status of a MS, region or even a farm, are presented in the EFSA report on HEIs for cattle meat inspection (EFSA, 2013).

Comparable data on the other proposed harmonised epidemiological indicators from the EU MSs were not available. This is because the indicators are in many cases quite specific, defining the animal population targeted as well as the specimen to be taken and the analytical method to be used.
CONCLUSIONS AND RECOMMENDATIONS

ToR 1: Define harmonised epidemiological criteria for specific hazards already covered by current meat inspection (trichinellosis, tuberculosis, cysticercosis, …) and for possible additional hazards identified in the Scientific Opinion on the hazards to be covered by inspection of meat (see Annex 1 of the mandate), which can be used to consider adaptations of meat inspection methodology (e.g. prevalence, status of infection).

Conclusions

- In this report harmonised epidemiological indicators (HEIs) are proposed for food-borne biological hazards related to domestic sheep and goats and meat thereof in the context of the Scientific Opinion on public health hazards to be covered by inspection of meat from sheep and goats (EFSA BIOHAZ Panel, 2013). These hazards include mycobacteria, which are already covered by meat inspection of small ruminants, as well as *T. gondii* and pathogenic VTEC, which were identified by the Scientific Opinion. An epidemiological indicator is understood to mean the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazards, such as audits of farms, that correlates with a human health risk caused by the hazard.

- The epidemiological indicators proposed in this report will provide relevant information to the risk managers (i.e. the European Commission and the Member States (MSs)), in order to consider whether adaptations in meat inspection methods may be relevant and to enable the MSs to carry out a risk analysis to support such decisions. The epidemiological indicators could be also used in future to help categorise countries, regions, slaughterhouses or potentially farms or herds/flocks, according to risk related to a particular hazard. Thus, the indicators could facilitate the implementation of risk-based meat inspection.

- The risk managers should decide on the most appropriate use of the epidemiological indicators. Depending on the purpose and the epidemiological situation of the country, the indicators may be applied at national, regional, slaughterhouse or farm/herd level and they can be used alone or in different combinations.

- The epidemiological indicator for *Toxoplasma* will enable the classification of animals into low or high risk at the slaughterhouse. A combination of several epidemiological indicators (i.e. auditing of controlled husbandry conditions at the farm, the age of the animals and serological testing at the slaughterhouse) has been proposed to increase the feasibility of the risk categorisation system.

- The epidemiological indicators for pathogenic VTEC can be used in the classification of slaughter batches according to the infection status related to the hazard at farm level. In addition, other indicators have been proposed to evaluate the measures taken in the slaughterhouses to control the hazard or to guarantee process hygiene.

- In cases of rare biological hazards in sheep and goat production, epidemiological indicators are suggested to enable surveillance for possible emergence of such hazards. This is the case for *Mycobacterium*.

- Today, the majority of small ruminants are raised outdoors, and a considerable proportion are moved between premises during their lifetime. This limits the use of auditing of controlled husbandry conditions as an epidemiological indicator.

- The data accumulated from the implementation of the HEIs will provide historical information over time on the infection status of the animals, farms and slaughterhouses. This information will be useful for the risk categorisation of farms, slaughterhouses and areas regarding their status. Where there is a history of negative test results, the information can also be used to reduce the testing frequency applied for HEIs.
The epidemiological indicators suggested for sheep and goats address risks at region, at farm and at slaughterhouse level using a variety of methods. The proposed HEIs are summarised in Table 4.

**Table 4:** Proposed harmonised epidemiological indicators for sheep and goats

<table>
<thead>
<tr>
<th>Indicators (animal/food category/other)</th>
<th>Food chain stage</th>
<th>Analytical/diagnostic method</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxoplasma gondii</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEI 1: Farms with controlled husbandry conditions</td>
<td>Farm</td>
<td>Auditing</td>
<td>Not applicable</td>
</tr>
<tr>
<td>HEI 2: Information on the age of the animals</td>
<td>Slaughterhouse</td>
<td>Food chain information</td>
<td>Not applicable</td>
</tr>
<tr>
<td>HEI 3: Detection of <em>T. gondii</em> infection</td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Blood</td>
</tr>
<tr>
<td>HEI 4: Detection of <em>T. gondii</em> infection in older animals (more than one year) from farms with controlled husbandry conditions</td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Blood</td>
</tr>
<tr>
<td>HEI 5: Absence of <em>T. gondii</em> infection in younger animals (less than one year) from farms without controlled husbandry conditions</td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Blood</td>
</tr>
<tr>
<td><strong>Pathogenic VTEC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEI 1: Occurrence of pathogenic VTEC in slaughter batch/group of animals one month before slaughter</td>
<td>Farm</td>
<td>Microbiology</td>
<td>Pooled faecal samples</td>
</tr>
<tr>
<td>HEI 2: Occurrence of pathogenic VTEC on fleece/pelt samples (after bleeding and before fleece/pelt removal)</td>
<td>Slaughterhouse</td>
<td>Microbiology</td>
<td>Fleece sample/pelt swab</td>
</tr>
<tr>
<td>HEI 3: Occurrence of pathogenic VTEC on carcases pre-chilling</td>
<td>Slaughterhouse</td>
<td>Microbiology</td>
<td>Carcase swabs</td>
</tr>
<tr>
<td>HEI 4: Occurrence of pathogenic VTEC on carcases post-chilling</td>
<td>Slaughterhouse</td>
<td>Microbiology</td>
<td>Carcase swabs</td>
</tr>
<tr>
<td><strong>Mycobacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEI 1: Official bovine tuberculosis status</td>
<td>Farm/region/Member State</td>
<td>Official records, food chain information</td>
<td>Not applicable</td>
</tr>
<tr>
<td>HEI 2: Human-pathogenic mycobacteria in sheep and/or goats at slaughter</td>
<td>Slaughterhouse</td>
<td>Visual meat inspection and Microbiology</td>
<td>Suspected lesions</td>
</tr>
</tbody>
</table>
Recommendations

- Whenever the small ruminant husbandry practices change towards more location-stable and intensive practices, it is recommended to review whether an indicator on controlled husbandry conditions can be applied for additional hazards.

- It is recommended that the MSs report the data generated from implementation and monitoring of the indicators within the framework of annual reporting in accordance with Directive 2003/99/EC.

- It is recommended that the potential indicators left out of this report owing to data gaps and lack of evidence are reviewed and their inclusion considered if more evidence is obtained. This would include, for example, the use of vaccine that is effective in reducing the presence of *T. gondii* cysts in meat, the effect of removing certain husbandry risk factors, and the effectiveness of process hygiene to avoid cross-contamination with pathogenic VTEC in slaughterhouses.

- The HEIs proposed by this report should be reviewed regularly in light of new information and the data generated from monitoring of them.

**ToR 2**: Provide a summary of comparable data from Member States based on the above-defined harmonised epidemiological criteria, if existing (e.g. from ongoing monitoring in humans, food or animals).

**Conclusions**

- The only available comparable data from the EU MSs are those related to one of the two HEIs proposed for mycobacteria, which refers to the official bovine tuberculosis status. No comparable data are available for the remaining proposed epidemiological indicators.

**ToR 3**: Recommend methodologies and minimum monitoring/inspection requirements to provide comparable data on such harmonised epidemiological criteria, in particular if comparable data are missing. These criteria should also be achievable in small MSs.

**Conclusions**

- For each epidemiological indicator the key elements of minimum monitoring or inspection requirements are defined. This includes the animal population to be targeted, the stage of the food chain at which the sampling should take place, type and details of the specimen to be taken, diagnostic or analytical method to be used, and a case definition.

**Recommendations**

- It is recommended that monitoring at any stage is designed to be epidemiologically sound with clearly stated objectives and acceptable levels of uncertainty.
REFERENCES


Dubey JP, 1996. Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. Veterinary Parasitology, 64, 65-70.


EFSA (European Food Safety Authority), 2007b. Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on monitoring of verotoxigenic Escherichia coli (VTEC) and identification of human pathogenic VTEC types. The EFSA Journal 2007, 579, 1-61.

EFSA (European Food Safety Authority), 2009a. Technical specifications for the monitoring and reporting of verotoxigenic Escherichia coli (VTEC) on animals and food (VTEC surveys on animals and food) on request of EFSA. EFSA Journal 2009; 7(11):1366, 43 pp. doi:10.2903/j.efsa.2009.1366


Epidemiological indicators for meat inspection of domestic sheep and goats


Katzer F, Brulisauer F, Collantes-Fernandez E, Bartley PM, Burrells A, Gunn G, Maley SW, Cousens C and Innes EA, 2011. Increased *Toxoplasma gondii* positivity relative to age in 125 Scottish sheep flocks; evidence of frequent acquired infection. Veterinary Research, 42.


seroprevalence in Dutch sheep using mixture models. Preventive Veterinary Medicine, 96, 232-240.


UK Agriculture, online. Background information on the stratification of the sheep industry in UK. Available online: http://www.ukagriculture.com/livestock/sheep_industry.cfm


APPENDICES

Appendix A. Food chain, risk and risk-reducing factors, possible human health epidemiological indicators and their evaluation

*Toxoplasma gondii*

I. Identification of potential epidemiological indicators

**Table 5:** Risk factors and potential epidemiological indicators for *Toxoplasma gondii* in small ruminants

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Farm (including contribution from wildlife)</th>
<th>Availability of data</th>
<th>Data availability to divide population into groups between which the risk varies</th>
<th>Suggested epidemiological indicator (HEI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor 1</td>
<td>Age of the sheep/goat – higher prevalence in older sheep/goat</td>
<td>These data should be available as part of FCI</td>
<td>Data available for different ages of animals and risk of exposure</td>
<td>Information on age of the animals at slaughter</td>
</tr>
<tr>
<td>Risk factor 2</td>
<td>Presence of cats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor 3</td>
<td>Outdoor access, grazing</td>
<td>Some data from research studies are available</td>
<td>Very few data available for different types of production systems or ages of animals but it is possible to obtain this information.</td>
<td>Farms with controlled husbandry conditions</td>
</tr>
<tr>
<td>Risk factor 4</td>
<td>Contamination of bedding, water and feedstuff with <em>T. gondii</em> oocysts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor 5</td>
<td>Absence of vaccination</td>
<td>Some data from research studies might be available</td>
<td>No data available</td>
<td>Vaccine use at the farm</td>
</tr>
<tr>
<td>Risk factor 6</td>
<td>Previous history of <em>T. gondii</em> infection in the farm</td>
<td>Some data from research studies might be available</td>
<td>No data available</td>
<td>Prior detection of <em>T. gondii</em> in abortion material, abortion storms due to <em>T. gondii</em> (submission of abortion material to diagnostic laboratories for necropsy and histological evaluation)</td>
</tr>
</tbody>
</table>

Table continued overleaf.
| Table 5 (continued). Risk factors and potential epidemiological indicators for *Toxoplasma gondii* in small ruminants |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| **Availability of data** | **Data availability to divide population into groups between which the risk varies** | **Suggested epidemiological indicator (HEI)** |
| Direct measurement of risk | Data on *T. gondii* in animals available from annual monitoring in the EU. | Some data are available | Detection of *T. gondii* infection (using different analytical methods) in: |
| Presence of *T. gondii* in the batch or the animal/carcase | | | o sheep and goats from all farms, except vaccinated animals |
| | | | o sheep and goats older than one year from farms with controlled husbandry conditions |
| | | | o sheep and goats younger than one year, from farms without controlled husbandry conditions |

**Transport to slaughterhouse**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Slaughterhouse</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Processing of meat and products thereof**

| Protective factor 1 | Heat treatment, freezing, salting, curing | - | - |
| Risk factor 1 | Combining meat from *T. gondii* positive and negative animals (e.g. through mincing) | - | - |

**Retail**

| Protective factor 1 | freezing the meat | - | - |

**Consumer**

| Risk factor 1 | Eating raw or undercooked meat | - | - |
| Risk factor 2<sup>(a)</sup> | Pregnancy/immunocompromised | - | - |

(a): Risk factor for severe outcome
### II. Evaluation of suggested indicators

**Table 6:** Suggested epidemiological indicators for *Toxoplasma gondii* in small ruminants

<table>
<thead>
<tr>
<th>Weighting factor</th>
<th>Indicators (animal/food category/other)</th>
<th>Food chain stage</th>
<th>Analytical/diagnostic method</th>
<th>Specimen</th>
<th>Quality of indicator(^{(a)}) ((0,1,2)^{(e)})</th>
<th>Appropriateness of indicator(^{(b)}) ((0,1,2)^{(c)})</th>
<th>Data availability(^{(c)}) ((0,1,2)^{(c)})</th>
<th>Feasibility(^{(d)}) ((0,1,2)^{(g)})</th>
<th>Total points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Information on the age of the animals</td>
<td>Slaughterhouse</td>
<td>FCI</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Detection of <em>T. gondii</em> infection</td>
<td>Farm</td>
<td>Serology</td>
<td>Blood</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Blood</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Meat juice</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slaughterhouse</td>
<td>PCR</td>
<td>Tissue (100g of heart)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Prior detection of <em>T. gondii</em> in abortion material</td>
<td>Farm</td>
<td>Histology</td>
<td>Tissue</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Farms with controlled husbandry conditions(^{(f)})</td>
<td>Farm</td>
<td>Auditing</td>
<td>Not applicable</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>Detection of <em>T. gondii</em> in animals older than one year from farms with controlled husbandry conditions(^{(g)})</td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Blood</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Absence of <em>T. gondii</em> in animals younger than one year from farms with non-controlled husbandry conditions(^{(h)})</td>
<td>Slaughterhouse</td>
<td>Serology</td>
<td>Blood</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).

(b): Appropriateness of indicator = how well the indicator correlates with human health risk caused by the hazard and/or the possibility/need to amend the meat inspection method.

(c): Data availability = are there data already available or is it easy to get the data needed?

(d): Feasibility = how laborious is the sampling and testing procedure?

(e): 0 = bad, 1 = moderate, 2 = good.

(f): As described in Appendix C.

(g): Young animals from these farms are assumed to pose very low risk.

(h): Breeding animals from these farms are assumed to pose high risk; therefore it is not necessary to test them.
Verocytotoxin-producing *Escherichia coli*

### I. Identification of potential epidemiological indicators

<table>
<thead>
<tr>
<th><strong>Table 7:</strong> Risk factors and potential epidemiological indicators for pathogenic VTEC in small ruminants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Farm (including contribution from wildlife)</strong></td>
</tr>
<tr>
<td><strong>Risk factor 1</strong></td>
</tr>
<tr>
<td>Farming practices that can lead to the introduction of VTEC (i.e. introduction of new animals, mixing with other flocks/herds, access to pasture, access to surface water, storage conditions of feed, possible contact with animals and wildlife)</td>
</tr>
<tr>
<td>Data available from research</td>
</tr>
<tr>
<td>Data available from audits of farms</td>
</tr>
<tr>
<td>Farms with controlled husbandry conditions</td>
</tr>
<tr>
<td><strong>Risk factor 2</strong></td>
</tr>
<tr>
<td>Farming practices that can lead to transmission of VTEC on the farm (i.e. animal density, wet bedding, unhygienic slurry practices, age mixing)</td>
</tr>
<tr>
<td>Data available from research</td>
</tr>
<tr>
<td>It is possible to obtain such data. There is no routine monitoring at present.</td>
</tr>
<tr>
<td>Occurrence of pathogenic VTEC in slaughter batch/group of animals one month before slaughter</td>
</tr>
<tr>
<td><strong>Risk factor 3</strong></td>
</tr>
<tr>
<td>Shedding of VTEC in pre-slaughter animals (batch level)</td>
</tr>
<tr>
<td>Data available from research</td>
</tr>
<tr>
<td>It is possible to obtain such data</td>
</tr>
<tr>
<td>Occurrence of pathogenic VTEC in slaughter batch/group of animals one month before slaughter</td>
</tr>
<tr>
<td><strong>Transport to slaughterhouse</strong></td>
</tr>
<tr>
<td><strong>Risk factor 1</strong></td>
</tr>
<tr>
<td>Loading and transport—cross-contamination, cleanliness of transport vehicle</td>
</tr>
<tr>
<td>Data available from research and studies on impact of transport on VTEC prevalence</td>
</tr>
<tr>
<td>It is possible to obtain such data</td>
</tr>
<tr>
<td>Pathogenic VTEC contamination of transport vehicles (microbiology on transport vehicles)</td>
</tr>
<tr>
<td>Audit of transport conditions (animal density, mixing of animals, sanitary conditions of vehicle, measurement of time of transport)</td>
</tr>
</tbody>
</table>

Table continued overleaf.
### Table 7 (continued). Risk factors and potential epidemiological indicators for pathogenic VTEC in small ruminants

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Slaughterhouse</th>
<th>Slaughterline</th>
<th>Processing of meat and products thereof</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lairage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor 1</td>
<td>Cross-contamination (mixing of animals from different origins, cleanliness of lairage)</td>
<td>Data available from research and studies on impact of lairage on VTEC prevalence in ovine animals</td>
<td>It is possible to obtain such data</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Slaughterline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor 3</td>
<td>VTEC contamination on fleece/pelt</td>
<td>Data available from literature</td>
<td>It is possible to obtain such data</td>
</tr>
<tr>
<td>Risk factor 4</td>
<td>Contamination of carcases during dressing and evisceration (including cross contamination)</td>
<td>Data available from literature</td>
<td>It is possible to obtain such data</td>
</tr>
<tr>
<td><strong>Processing of meat and products thereof</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor 1</td>
<td>Cross-contamination during processing</td>
<td>Data available from literature and from national surveillance/ monitoring</td>
<td>It is possible to obtain such data</td>
</tr>
<tr>
<td><strong>Retail</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor 1</td>
<td>Temperature abuses</td>
<td>Should be available from hazard analysis and critical control point programmes</td>
<td>It is possible to obtain such data (temperatures above 12 °C are considered high risk for VTEC growth)</td>
</tr>
<tr>
<td>Risk factor 2</td>
<td>Cross-contamination at retail</td>
<td>Some prevalence data available from literature and national surveillance/monitoring</td>
<td>It is possible to obtain such data</td>
</tr>
</tbody>
</table>

Table continued overleaf.
Table 7 (continued). Risk factors and potential epidemiological indicators for pathogenic VTEC in small ruminants

<table>
<thead>
<tr>
<th>Consumer</th>
<th>Availability of data</th>
<th>Data availability to divide population to groups between which the risk varies</th>
<th>Suggested epidemiological indicator (HEI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor 1</td>
<td>Handling in the kitchen and cross contamination</td>
<td>Data available from research</td>
<td>Data not available</td>
</tr>
<tr>
<td>Risk factor 2</td>
<td>Undercooking of ovine meat</td>
<td>Data available from research</td>
<td>Data not available</td>
</tr>
<tr>
<td>Risk factor 3</td>
<td>Temperature abuses</td>
<td>Data available from research</td>
<td>Data not available</td>
</tr>
</tbody>
</table>
II. Evaluation of suggested indicators

Table 8: Suggested epidemiological indicators for pathogenic VTEC in small ruminants

<table>
<thead>
<tr>
<th>Weighting factor</th>
<th>Indicators (animal/food category)</th>
<th>Food chain stage</th>
<th>Analytical/diagnostic method</th>
<th>Specimen</th>
<th>Quality of indicator(^{(a)}) ((0,1,2))(^{(e)})</th>
<th>Appropriateness of indicator(^{(b)}) ((0,1,2))(^{(e)})</th>
<th>Data availability(^{(c)}) ((0,1,2))(^{(e)})</th>
<th>Feasibility(^{(d)}) ((0,1,2))(^{(e)})</th>
<th>Total points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farms with controlled husbandry conditions</td>
<td>Farm Audit</td>
<td>Not applicable</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence of pathogenic VTEC in slaughter batch/group of animals one month before slaughter</td>
<td>Farm Microbiology Pooled faecal samples</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathogenic VTEC contamination of transport vehicles and lairage</td>
<td>Transport and lairage Microbiology Environmental swabs</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport and lairage hygiene</td>
<td>Transport and lairage Auditing Not applicable</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual inspection of incoming animals (clean animal scoring system)</td>
<td>Slaughterhouse Visual inspection Not applicable</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence of pathogenic VTEC on fleece/pelt samples (after bleeding and before fleece/pelt removal)</td>
<td>Slaughterhouse Microbiology Fleece / Pelt sample</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence of pathogenic VTEC on carcases pre-chilling</td>
<td>Slaughterhouse Microbiology Carcase swab</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occurrence of pathogenic VTEC on carcases post-chilling</td>
<td>Slaughterhouse Microbiology Carcase swab</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1.55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with human health risk caused by the hazard and/or the possibility/need to amend the meat inspection method.
(c): Data availability = are there data already available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure and how much would the sampling/testing cost or are the data already available (no additional sampling/testing needed)?
(e): 0 = bad, 1 = moderate, 2 = good.
Mycobacteria

I. Identification of potential epidemiological indicators

Table 9: Risk factors and potential epidemiological indicators for mycobacteria in domestic sheep

<table>
<thead>
<tr>
<th>Farm (including contribution from wildlife)</th>
<th>Availability of data</th>
<th>Data availability to divide population into groups between which the risk varies</th>
<th>Suggested epidemiological indicator (HEI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor 1: Contact with infected animals (cattle and/or wildlife)</td>
<td>Data available from official reports and in the literature.</td>
<td>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MSs and to substantiate freedom from disease as well as maintenance of the status</td>
<td>Official bovine tuberculosis status of the farm/region/country of origin</td>
</tr>
<tr>
<td>Risk factor 2: Outdoor production systems with limited biosecurity (mixed grazing, unrestricted access by susceptible wildlife)</td>
<td>Data available from official reports and in the literature.</td>
<td>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MSs and to substantiate freedom from disease as well as maintenance of the status</td>
<td>Controlled husbandry conditions</td>
</tr>
</tbody>
</table>

Transport to slaughterhouse

Slaughterhouse

Risk factor 1: Ability to recognise and report granulomatous lesions for further laboratory investigation

Processing of meat and products thereof

Retail

Consumer
Table 10: Risk factors and potential epidemiological indicators for mycobacteria in goats

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Availability of data</th>
<th>Data availability to divide population into groups between which the risk varies</th>
<th>Suggested epidemiological indicator (HEI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm (including contribution from wildlife)</td>
<td>Data available from official reports and in the literature.</td>
<td>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</td>
<td>Official bovine tuberculosis status</td>
</tr>
<tr>
<td><strong>Risk factor 1</strong></td>
<td><strong>Data available from official reports and in the literature.</strong></td>
<td><strong>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</strong></td>
<td><strong>Official bovine tuberculosis status</strong></td>
</tr>
<tr>
<td>Contact with infected animals (cattle and/or wildlife, where <em>M. bovis</em> endemic)</td>
<td>Data available from official reports and in the literature.</td>
<td>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</td>
<td>Controlled husbandry conditions</td>
</tr>
<tr>
<td><strong>Risk factor 2</strong></td>
<td><strong>Data available from official reports and in the literature.</strong></td>
<td><strong>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</strong></td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td>Outdoor production systems with limited biosecurity (mixed grazing, unrestricted access by susceptible wildlife)</td>
<td>Limited prevalence data available from official reports and in the literature</td>
<td>Controlled husbandry conditions and biosecurity</td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td><strong>Risk factor 3</strong></td>
<td><strong>Data available from official reports and in the literature.</strong></td>
<td><strong>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</strong></td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td>Contact with infected livestock, particularly goats, where <em>M. caprae</em> is endemic</td>
<td>Limited prevalence data available from official reports and in the literature</td>
<td>Controlled husbandry conditions and biosecurity</td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td><strong>Risk factor 4</strong></td>
<td><strong>Data available from official reports and in the literature.</strong></td>
<td><strong>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</strong></td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td>Presence of infected goats in the farm</td>
<td>Limited prevalence data available from official reports and in the literature</td>
<td>Controlled husbandry conditions and biosecurity</td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td><strong>Transport to slaughterhouse</strong></td>
<td><strong>Data available from official reports and in the literature.</strong></td>
<td><strong>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</strong></td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td><strong>Slaughterhouse</strong></td>
<td><strong>Data available from official reports and in the literature.</strong></td>
<td><strong>Prevalence data are available to identify the range of affected species, including wildlife, and affected regions/MS and to substantiate freedom from disease as well as maintenance of the status</strong></td>
<td>Controlled husbandry conditions and biosecurity</td>
</tr>
<tr>
<td>Direct measurement of risk</td>
<td>Data might be available from official reports</td>
<td>Data might be available from official reports</td>
<td>Human-pathogenic mycobacteria in sheep and/or goats at slaughter</td>
</tr>
<tr>
<td>Ability to recognise and report granulomatous lesions for further investigation</td>
<td>Data might be available from official reports</td>
<td>Data might be available from official reports</td>
<td>Human-pathogenic mycobacteria in sheep and/or goats at slaughter</td>
</tr>
<tr>
<td><strong>Processing of meat and products thereof</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Retail</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Consumer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
II. Evaluation of suggested indicators

Table 11: Suggested epidemiological indicators for mycobacteria in sheep

<table>
<thead>
<tr>
<th>Weighting factor</th>
<th>Food chain stage</th>
<th>Analytical/diagnostic method</th>
<th>Specimen</th>
<th>Quality of indicator&lt;sup&gt;(a)&lt;/sup&gt; (0,1,2)&lt;sup&gt;(c)&lt;/sup&gt;</th>
<th>Appropriateness of indicator&lt;sup&gt;(b)&lt;/sup&gt; (0,1,2)&lt;sup&gt;(c)&lt;/sup&gt;</th>
<th>Data availability&lt;sup&gt;(c)&lt;/sup&gt; (0,1,2)&lt;sup&gt;(c)&lt;/sup&gt;</th>
<th>Feasibility&lt;sup&gt;(d)&lt;/sup&gt; (0,1,2)&lt;sup&gt;(c)&lt;/sup&gt;</th>
<th>Total points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Official bovine tuberculosis status</td>
<td>Farm/region/Member State</td>
<td>Official records</td>
<td>Not applicable</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>Controlled husbandry conditions</td>
<td>Farm</td>
<td>Auditing</td>
<td>Not applicable</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.85</td>
</tr>
<tr>
<td>Human-pathogenic mycobacteria in sheep and/or goats at slaughter</td>
<td>Slaughterhouse</td>
<td>Visual meat inspection and microbiology</td>
<td>Suspected lesions</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with human health risk caused by the hazard and/or the possibility/need to amend the meat inspection method.
(c): Data availability = are there already data available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure?
(e): 0 = bad, 1 = moderate, 2 = good.
### Table 12: Suggested epidemiological indicators for mycobacteria in domestic goats

<table>
<thead>
<tr>
<th>Weighting factor</th>
<th>Indicators (animal/ food category)</th>
<th>Food chain stage</th>
<th>Analytical/ diagnostic method</th>
<th>Specimen</th>
<th>Quality of indicator $^{(e)}$</th>
<th>Appropriateness of indicator $^{(e)}$</th>
<th>Data availability $^{(e)}$</th>
<th>Feasibility $^{(e)}$</th>
<th>Total points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Official bovine tuberculosis status</td>
<td>Farm/region/ Member State</td>
<td>Official records</td>
<td>Not applicable</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Controlled conditions at the farm for goats, including biosecurity</td>
<td>Farm</td>
<td>Audit</td>
<td>Not applicable</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Detection of infection in live goats</td>
<td>Farm</td>
<td>Single Intradermal Comparative Cervical Tuberculin (SICCT) test</td>
<td>Live goat</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Detection of infection in live goats</td>
<td>Farm</td>
<td>Gamma Interferon tuberculosis blood test</td>
<td>Blood</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Human-pathogenic mycobacteria in sheep and/or goats at slaughter</td>
<td>Slaughterhouse</td>
<td>Visual meat inspection and microbiology $^{(g)}$</td>
<td>Suspected lesions</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

(a): Quality of indicator = how reliable the data for the indicator would be (e.g. test sensitivity).
(b): Appropriateness of indicator = how well the indicator correlates with human health risk caused by the hazard and/or the possibility/need to amend the meat inspection method.
(c): Data availability = are there already data available or is it easy to get the data needed?
(d): Feasibility = how laborious is the sampling and testing procedure?
(e): 0 = bad, 1 = moderate, 2 = good.
(f): Quality of results is influenced by the time between sampling and laboratory testing.
(g): Detection and identification of the agent from lesions detected through visual inspection (culture and/or PCR).
Appendix B. Correlation between detection of *Toxoplasma gondii* antibodies and presence of tissue cysts in small ruminants

In sheep, and probably also in goats, both antibodies against *T. gondii* as well as tissue cysts persist (Dubey, 2009). Therefore, a strong correlation between detection of antibodies against *T. gondii* and presence of tissue cysts can be expected, although studies that directly compare presence of antibodies and detection of tissue cysts are limited. *T. gondii* was isolated by cat or mouse bioassay from 53 out of 68 seropositive lambs, and pooled heart samples from 44 seronegative lambs remained negative in a cat bioassay (Dubey et al., 2008). In addition, a study carried out by Opsteegh et al. (2010a) showed a strong correlation between antibody responses and parasite detection by magnetic capture and PCR (Opsteegh et al., 2010b). Nonetheless, there are two other studies in which many seropositive sheep remained negative by bioassay. *T. gondii* was isolated by bioassay from only 8 out of 30 heart samples (50 g) collected from seropositive ewes in one study (Dumetre et al., 2006), and seroprevalence (15 % in lambs and 81 % in sheep over 12 months) was much higher than prevalence of detection by mouse bioassay (2 % in lambs, and 42 % in sheep over 12 months old) in another study (Halos et al., 2010). In goats, the relation is similar. Two out of 69 seronegative goats and 27 out of 43 seropositive goats tested positive by bioassay on 50 g of heart in 5 mice (Dubey et al., 2011).

Discrepancies between antibody and tissue cyst detection, not due to analytical errors, may occur when:

- Parasites can be detected in seronegative animals in the acute phase of infection, when the antibody response has not yet developed. Experimental infections in sheep have shown IgG seroconversion at 12–14 days post infection or later depending on the serological assay used (Dubey, 2009) while parasites can be detected in blood between three and 10 days post infection (Esteban-Redondo and Innes, 1998).
- Congenital transmission in successive litters has been demonstrated in mice, and sometimes these mice do not develop an antibody response (Owen and Trees, 1998; Rejmanek et al., 2010), which is suggestive of immune tolerance. Congenital transmission has also been shown in subsequent lambings, but only by PCR (Duncanson et al., 2001; Williams et al., 2005; Morley et al., 2008; Hide et al., 2009), and not by histological examination or serology (Rodger et al., 2006; Dubey, 2009). Mason et al. (2010) used PCR and serology on the same lambs and did not find a correlation between PCR-based detection in umbilical cord and serology at four months of age (Mason et al., 2010). Although it is unclear whether PCR-based detection of *T. gondii* in birth material means that lambs carry infectious tissue cysts, the possibility of congenitally infected lambs that carry tissue cysts but remain seronegative needs to be further investigated, as it would decrease the value of serological tests in monitoring for the risk of human infection.
- Detection of antibodies without presence of tissue cysts is likely in vaccinated animals (especially in ewes from the United Kingdom, which are potentially vaccinated).
- In naturally infected seropositive animals, tissue cysts are likely to remain undetected if only a small tissue sample is tested, as tissue cysts are present at a low concentration that may vary between different tissues (<1 per 50 g has been suggested (Dubey et al., 1996)). This explains the difference between sensitivity of bioassay in cats (up to 500 g of tissue) and in mice (50–100 g), as well as the higher detection rates when testing predilection sites compared with other tissues.

In conclusion, a positive result in a serological test gives a strong indication that the sheep or goat carries tissue cysts and poses a risk of infection to consumers. A sheep or goat with a negative result in a serological test can still be in the acute phase of infection, so this does not exclude all risk to human consumers. Because it is unlikely that many animals are in this phase of infection, which lasts about two weeks, the delay in antibody response does not substantially affect the value of serological tests as a tool to get an indication of the infection level of a batch or flock. If congenital transmission from
chronically infected sheep occurs as commonly as suggested by the PCR-based studies and often results in immunotolerance, this would decrease the value of serological tests in determining the infection level in a flock or batch, or certain flocks (if related to a genetic predisposition in the sheep). However, currently most evidence suggests that congenital transmission from chronically infected sheep is not that common.
Appendix C. Proposed requirements for controlled husbandry conditions on farms regarding *Toxoplasma gondii*

Throughout this scientific report references to controlled husbandry conditions have been made and it will be beneficial to have a common understanding of what could be considered adequate requirements for small ruminants in this context.

The majority of sheep and goats are kept outdoors and, unlike other livestock species, a large proportion of small ruminant flocks/herds in the EU are regularly relocated according to weather and feeding availability. For controlled husbandry to effectively reduce the risks of *T. gondii*, the herd/flock must be protected from all or most of the relevant risk factors. This is likely to be possible only in a very small proportion of small ruminant farming systems today (e.g. dairy goats that are kept indoors their whole life). In this report, generic requirements for controlled husbandry conditions are provided, even if they apply only to a small number of herds/flocks. However, good husbandry practices should apply to all keeping of small ruminants independently of the ability to comply with controlled husbandry requirements.

The main objective of implementing controlled husbandry conditions adapted to specific production systems is to minimise animal health and food safety risks arising at the farm level during animal production with potential consequential risks for humans (OIE and FAO, 2008). As a general principle, closed farming systems and all-in–all-out systems are the best ones from a food safety and biosecurity point of view but, as stated at the beginning of this section, this is not always possible and depends on the production system used.

Controlled husbandry conditions differ depending on the hazards that could affect livestock production. As controlled husbandry conditions are proposed as an indicator only for *T. gondii* in small ruminants, the following measures specifically aim to reduce exposure to *T. gondii*:

- Livestock is not allowed outdoor access.
- No access of cats to livestock housing.
- Feed and bedding material is protected from cats, wildlife and vermin.
- Drinking water must be delivered in a closed system and not originate from surface water without proper treatment that would ensure the elimination of *T. gondii* oocysts.
- Boots should be provided for exclusive use inside the livestock house to prevent contaminated soil being brought in.
- Birthing policy should include proper disposal of birth materials and recording of abortion history. Suspected infectious abortions should be sent for diagnosis and a record of diagnoses should be kept.
- There should be a policy of *T. gondii* testing of any animals introduced into the herd/flock or sourcing from herds/flocks free from *T. gondii*.
- Training of farm staff should also be part of the controlled housing conditions to ensure that staff are fully aware of the biosecurity standards and good farming/hygiene practices.
- Historical record of stable and consistent practice in the same location, which continue to exclude the risk of introduction of *T. gondii*. 
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>BIOHAZ</td>
<td>Biological Hazards</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>CVO</td>
<td>Chief Veterinary Officer</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>ECDC</td>
<td>European Centre for Disease Prevention and Control</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>HEI</td>
<td>Harmonised Epidemiological Indicator</td>
</tr>
<tr>
<td>HUS</td>
<td>Haemolytic Uraemic Syndrome</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Gamma interferon</td>
</tr>
<tr>
<td>MS</td>
<td>Member State</td>
</tr>
<tr>
<td>MAA</td>
<td><em>Mycobacterium avium</em> subsp. <em>avium</em></td>
</tr>
<tr>
<td>MAC</td>
<td><em>Mycobacterium avium</em> complex</td>
</tr>
<tr>
<td>MAP</td>
<td><em>Mycobacterium avium</em> subsp. <em>paratuberculosis</em></td>
</tr>
<tr>
<td>MTC</td>
<td><em>Mycobacterium tuberculosis</em> complex</td>
</tr>
<tr>
<td>NTM</td>
<td>Non-tuberculous mycobacteria</td>
</tr>
<tr>
<td>OTF</td>
<td>Officially Tuberculosis Free</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>SICCT</td>
<td>Single Intradermal Comparative Cervical Tuberculin</td>
</tr>
<tr>
<td>ToR</td>
<td>Terms of Reference</td>
</tr>
<tr>
<td>VTEC</td>
<td>Verocytotoxin-producing <em>Escherichia coli</em></td>
</tr>
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</table>