2. LITERATURE REVIEW

2.1 Morphology and development of the genus *Trichinella*

Trichinella nematodes belong to the family Trichuridae and are characterized by a length of 1-7 mm, a cylindrical and tapered anterior and posterior ends. The elongated esophagus contains prominent stichocytes and the bacillary bands (Kozek, 2005). Female worms are about twice of the length of males (1.4-1.6 mm) with a similarly located anus. The single uterus is filled with developing eggs in its posterior portion whereas the anterior portion contains fully developed hatching juveniles (Soulsby, 1982). The females are viviparous, laying first larvae and then dying shortly after the completion of the oviposition (Corwin and Stewart, 1999).

In 1835 James Paget has discovered the *Trichinella* parasite from the human muscle and in 1846; Joseph Leidy has first reported it from the swine muscle, referred by Gould (1970a). The German scientists Leuckart, Virchow and Zenker in 1860 have first elucidated the mechanism of the infection and the life cycle as referred by Campbell (1983) and Oivanen (2005). The pivotal events that led to the current taxonomy of *Trichinella* were the experimental attempts to infect laboratory rodents and pigs with isolates from wild animals (Murrell *et al.*, 2000).

According to the zoological classification of the taxonomy of the genus *Trichinella* it belongs to the phylum Nematoda, the class Adenophorea, the order Trichinellida and the super family Trichinelloidea (Noble *et al.*, 1989). But based on results from the ribosomal deoxyribonucleic acid sequences, two classes, Secernentea and Adenophorea, are referred (Blaxter *et al.*, 1998). Today, two main clades are recognized in the genus *Trichinella,* one that encompasses species that encapsulate in the host tissue and a second that does not encapsulate following the muscle cell differentiation. The species and genotypes of the first clad parasitize only in mammals, whereas of the three species that comprise the second clad infects mammals, birds and reptiles (Pozio and Zarlenga, 2005).

2.2 Life cycle

All members of the genus *Trichinella* have a direct life cycle where both adult and larval stages occur in the same host (Kassai, 1999; Bowman *et al.,* 2003), as outlined in figure 1. The infection is passed from host to host through ingestion of infective L1 (first stage) larvae in muscle tissue. The L1 larvae are enclosed in the muscle cells of the previous host. During the digestion process in the recipient host the infective L1 larvae are released into the small intestine. The development of the first to the fifth pre adult larval stages (L1-L5) in the epithelium of the small intestine is rapid and takes only 30 hours (Despommier, 1983; Kociecka *et al.,* 2003). Males die soon after copulation and mature females release about 1500 L1 larvae into the lymph spaces of the small intestine from 5-6 days onward for up to the next two months. The newborn larvae enter the lymphatics, mesenteric veins and proceed to migrate through the arterial circulation for a further 7-8 days as they mature and develop until the larvae are capable of invading the skeletal muscle (Straw *et al.,* 1999). Once in skeletal muscle the larvae enter the muscle cells where they mature further into infective L1 larvae during the first 2-3 weeks (Bowman *et al.,* 2003).

The larva absorbs the nutrients from the muscle cells and increases its length to 1 mm. It finally coils and remains dormant. This alteration in the muscle cell induces to create a ‘nurse cell with or without capsule’ from the original muscle cell. There the larva receives nutrients and remains viable inside the host for many years. The development cycle of a nurse cell after post infection varies depending on the *Trichinella* spp. For *T. spiralis* it is 16 days, for *T. nativa* 20-30 days, for *T. nelsoni* 34 days and for *T. murrelli* 60 days (Näreaho, 2006). The differences in the encapsulation times are probably the result of different maturation and reproduction rates since the capsule becomes apparent around day nine after the arrival of the newborn larvae to the muscles (Li and Ko, 2001). Once in muscle *Trichinella* may survive for several years until ingested by the next host (Kociecka *et al.,* 2003). The living larvae of *Trichinella spiralis* have been found 39 years after the infection in a human (Fröscher *et al.,* 1988) and it has been assumed that even after the death of the

host the parasite remains infective for weeks or even longer (Despommier *et al.*, 1991).

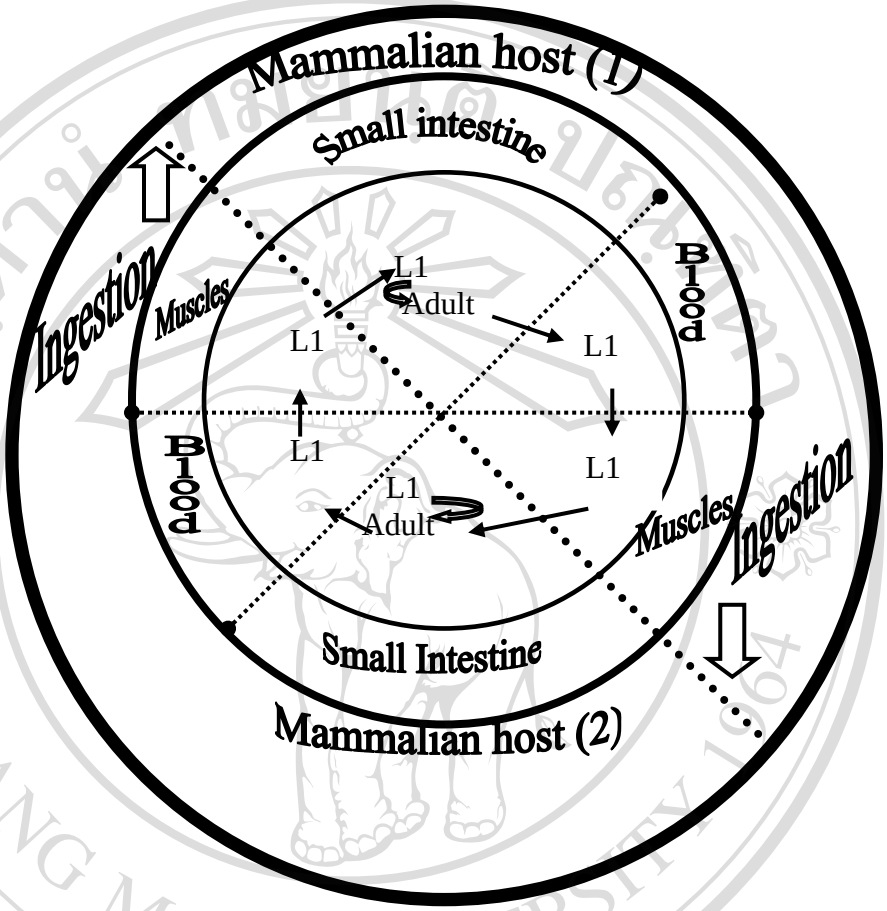

Figure 1: Diagram showing the direct life cycle of *Trichinella* (Adapted from Kassai, 1999)

2.3 Different *Trichinella* species and their epidemiology

From 1835 to the middle of the 20th century it was commonly assumed that all cases of trichinellosis were caused by a single species, *Trichinella spiralis* (Murrell *et al.*, 2000). Today, a total of 11 genotypes of *Trichinella* distributed by climate zones or by host species are identified (Pozio *et al.*, 2002). Eight were recognized at the species level like *T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. murrelli, T. nelsoni, T. papua*e and *T. zimbabwensis* whereas three (T6, T8 and T9) were only defined at an uncertain taxonomic level (Pozio *et al.*, 2001b; Kurdova *et al.*, 2004).

The studies on *Trichinella* have shown that the host range is wider with an extensive geographical distribution (Bolas-Fernandez and Wakelin, 1989; Pozio, 2005). Three classes of vertebrates (mammals, birds and reptiles) are known to act as hosts and even fishes are reported to be paratenic hosts (Moretti *et al.*, 1997). The epidemiology of the *Trichinella* infection varies greatly by the species depending on the diet, the life span, the distribution and the relationship to humans (Pozio, 2005).

Trichinella spiralis shows the worldwide distribution. The predominant hosts of this species are domestic and sylvatic swine, horse, brown rat, cat, dog and a broad range of sylvatic carnivores (Pozio, 2001a; Pozio, 2005). *Trichinella* produces more than 90 newborn larvae whereas the other species produce less than 60 newborn larvae per 72 hours (Pozio *et al.*, 1992). *Trichinella spiralis* is specifically characterized by six unique allozyme banding patterns generated by ACP, ALAT, EST, GLDH, PGM and SOD (Murrell *et al.*, 2000). It is the etiological agent of most of the human infections and deaths around the world.

Trichinella nativa belongs to the sylvatic cycle. It was documented in the frigid zones of Asia, North America and Europe. This species has limited infectivity for swine and rat but is commonly found in wild canids, bears and walruses. The muscle larvae of this species are high resistant to freezing (Pozio *et al.*, 1994). It was reported that they would survive -18^{0}C temperature for up to 5 years (Dick and Pozio, 2001). *Trichinella nativa* is uniquely characterized by two allozyme markers, ME and 6 PGD (Murrell *et al.*, 2000). Human infections were reported from frigid zones of Canada, Siberia, Greenland and Kamchatcka (Nelson *et al.*, 2003).

Trichinella britovi belongs to the sylvatic cycle and the larvae of this species are capable of surviving in frozen pig muscles for up to 3 weeks and up to 11 months in carnivore muscles (Dick and Pozio, 2001). It was reported from temperate areas of the polar region and also from the Iberian Peninsula to Kazakhstan, Iran and Turkey (Pozio, 2001a). It is likely that the distribution area encompasses other Asiatic countries (India and China) for which no information is currently available (Pozio and Zarlenga, 2005). The distinctive features of *Trichinella britovi* are its low infectivity

for rats, the greater resistance to freezing, the slow nurse cell development and the low in vitro production of newborn larvae (Murrell *et al.*, 2000). *Trichinella britovi* is identified by a single unique allozyme ACP (La Rosa *et al.*, 1992). It is predominantly reported in wild carnivores and occasionally in pigs and horses (Kapel and Gamble, 2000). Human infection from consumption of free ranging pig, game and horse meat from this species was documented in France, Italy, Spain and Turkey (Pozio *et al.*, 2001a). However the clinical course of disease is benign and no death has been reported yet (Pozio and La Rosa, 2003).

Trichinella pseudospiralis is a global species. It does not induce collagen capsule during the muscle phase of the infection. It was recovered from raptorial birds, wild carnivores, omnivores, rats and marsupials in Asia, North America and Europe and from the Australian subcontinent (Pozio, 2005; Pozio and Zarlenga, 2005). *Trichinella pseudospiralis* is identified by 12 unique allozymes ACP, ADA, ALAT, ALDO, GPD, LDH, EST, G6PD, GLDH, GOT, SOD and TPI (La Rosa *et al.*, 1992). The increasing reports of this species in domestic and sylvatic swine in Europe, the USA and in Southeast Asia show that it has a potential to infect humans.

Trichinella murrelli has been detected throughout the continental USA and Canada (Pozio *et al.*, 2001b). The infection of this species was documented in sylvatic carnivores living in the temperated areas of the Neoarctic region. This species has a low resistance to freezing and it is weak in its nurse cell development as well as in the newborn larvae production (Malakauskas and Kapel, 2003). It has recovered from wildlife and occasionally from horses but it has a very low infectivity for domestic pigs. Mostly humans get infected from game meat but horses are also identified as a source for a large outbreak in humans (Ancelle, 1998).

Trichinella nelsoni is the etiological agent of infection of sylvatic carnivores living in eastern Africa from Kenya to South Africa (Pozio and Zarlenga, 2005). In comparison to other species it has a greater resistance to elevated temperature. *Trichinella nelsoni* has a low infectivity for domestic pigs and rats but it has detected in bush pigs and warthogs (Pozio *et al.*, 1997; Kapel, 2001). This species is identified

by three unique allozymes ADA, GLDH and TPI (La Rosa *et al.*, 1992). The muscle larvae of this species may survive up to 56^0C for 60 minutes while the larvae of other species will die within 10 minutes at this temperature (Murrell *et al.*, 2000). This species shows low infectivity in humans. Only a single death was reported due to a high (>4000 larvae/g) larvae load of *T. nelsoni* (Bura and Willett, 1997).

Trichinella papuae is a non-encapsulating species of *Trichinella.* The length of *T. papuae* larvae is one third greater than that of *T. pseudospiralis.* It is very resistant to freezing (Murrell *et al.*, 2000) and can survive minus 5^0C storage temperature for 4 weeks (Webster *et al.*, 2002). It was detected only in Papua New Guinea and is able to infect both mammals and reptiles (Pozio and Zarlenga, 2005). The wild pigs are the most important reservoir of this species (Owen *et al.*, 2000) and serve as route of transmission to saltwater crocodiles and humans (Owen *et al.*, 2005).

Trichinella zimbabwensis is a non-encapsulating species of *Trichinella* and was detected in the farmed crocodiles of Zimbabwe and Ethiopia (Pozio *et al.*, 2002) as well as in sylvatic crocodiles of Mozambique (Pozio, 2005). Under laboratory conditions this species can infect domestic pigs, monkeys, rats, mice and foxes. Therefore it can be concluded that mammals are also a suitable host for this species. However, no naturally infected mammals are found yet (Pozio and Zarlenga, 2005).

2.4 Pathogenesis and clinical symptoms referring to *Trichinella spiralis* in the human

Trichinella infection of meat from food animals and games is important because of the risk of trichinellosis in humans who eat raw or undercooked meat (OIE, 2004). The larvae of *Trichinella* are present in the voluntary skeletal muscles of their hosts and the ingestion of these tissues shall transmit infections to a susceptible individual. It is estimated that a minimum infective dose of *Trichinella spiralis* for a human is 70-150 ingested larvae (EFSA, 2005). If ≥ 500 larvae are ingested a life-threatening condition will be reached (Murrell, 1985; Battelli *et al.*, 1994; Oivanen, 2005). The transplacental transmission of larvae has occurred in mouse and human,

but not in pig (Bowman *et al*., 2003). The person-to-person spread of the disease has not been reported yet (Urquhart *et al.*, 1996).

Trichinellosis continues to be a public health concern throughout the world. The experience from the past decade has shown a consistent increase of human cases (Murrell and Pozio, 2000). *Trichinella spiralis* is the etiological agent of most of the human infections and deaths around the world. That is because of its higher pathogenicity and a stronger immune reaction in humans (Gomez Morales *et al.*, 2002). The pathogenesis and the associated clinical symptoms in human beings from *Trichinella spiralis* infection can be described as follows:

Enteral phase: The enteral phase starts after consuming the contaminated meat. Most of the time people are asymptomatic. During the first week of the enteral phase moderate to severe symptoms like malaise, upper abdominal pain, mild transient diarrhea, nausea, vomiting and sub febrile temperature can develop in some patients (Hermanowska-Spakowicz *et al.*, 1993). Diarrhea is more persistent than vomiting and lasts up to 3 months, which causes severe dehydration. Together with enteritis this might be an occasional cause of death (Bruschi and Murrell, 2002).

Parenteral phase: The parenteral phase begins just after the larvae entered the circulation with the invasion of all organs lasting up to 3 to 4 weeks. The ocular signs like the edema of eyelids, chemosis, conjunctivitis, conjunctival hemorrhage, disturbed vision and blindness may develop in this phase. The periorbital edema is peculiar and ranges in 17-100% of the trichinellosis patients (Tassi *et al*., 1991). Cardiovascular disturbances and myocarditis are observed in 5-20% of all infected people (Dupouy-Camet *et al.*, 2002).

Muscle phase: The muscle phase starts with the entering of the larval in the striated skeletal muscles not before the fourth week. At this time the muscles (extra ocular, masseter, glossal, laryngeal, diaphragmatic, neck and intercostal) of the body usually become painful. The pain may be so severe that it limits the function of arms and legs, inhibits walking, speaking, moving the tongue, breathing and swallowing (Bruschi and Murrell, 2002). The muscles become edematous and the patient feels

weakness with prolonged progressive muscular hypertrophy (Chotmongkol *et al.*, 2005). This is associated with the invasion of the muscles by the migrating larvae which can damage muscle cells, directly or indirectly, with stimulating the infiltration of the inflammatory cells (Bruschi and Murrell, 2002). This causes an increase in the levels of muscle enzymes like creatine phosphokinase, lactate dehydrogenase, aldolase and occasionally asparatate aminotransferase and liver enzyme (Capo and Despommier, 1996; Jonwutiwes *et al.*, 1998; SCVPH, 2001).

Eosinophilia (1400-8700/cubic mm) is present in every case with leukocytosis (12500-18000/cubic mm) (Capo and Despommier, 1996; Dupouy-Camet *et al.*, 2002). Eosinophilia is significantly higher in patients with neurological complications (Fourestie *et al.*, 1993) and correlated with the degree of myalgia (Ferraccioli *et al.*, 1988; Kociecka, 2000). In this phase the central nervous system, the lungs, the kidneys and the skin might be affected (Wang *et al*., 2006).

The physicians only advance diagnosing the patient as being suspected of trichinellosis if the patient starts to show the trichinellotic syndrome, that is characterized by facial edema, muscle pain and swelling, weakness, fever (39-40^{0}C), anorexia, headache, conjunctivitis and urticaria (Pawlowski, 1983). However, the major complications of this infection are myocarditis and encephalitis, which are both life threatening and often present, simultaneously (Fourestie *et al.*, 1993). The cardiovascular complication symptoms include pain in the heart region, tachycardia and an abnormal electrocardiogram. The cardiovascular complications like thromboembolic disease, specifically deep thrombophlebitis, intraventricular thrombi and /or pulmonary embolism can lead to death (Pratesi *et al.*, 2006).

Previously it was assumed that the mechanical damage induced by the migrating larvae leads to cardiac symptoms (Gould, 1970a). The recent studies, however, have identified organ specific autoantibodies in trichinellosis patient sera (Pratesi *et al.*, 2006). Neurological complications were reported in 3-46% of the cases (Dupouy-Camet *et al.*, 2002). Such patients show unconsciousness, somnolence and apathy and some of them show signs of meningitis and encephalopathy (Feydy *et al.*, 1996).

2.5 Diagnostic measures in animals

The *Trichinella* infection in pigs and other species can be detected, directly or indirectly (OIE 3.5.3 Article; European Union 77/96/EEC Directive, European Council Regulation 807/2003). The direct demonstration is based on the detection of the parasitic larval stages in muscle tissues preferentially from the predilection sites, like diaphragm and tongue in swine (Serrano *et al.,* 1999; Kapel *et al.,* 2005), diaphragm, masseter and tongue in horse (Pozio *et al.,* 1999b; Theodoropoulos *et al.,* 2000; Kapel *et al.,* 2005) and lower limb, tongue and diaphragm in fox (Nöckler and Voigt, 1997). The indirect demonstration of the parasite can be done through serological and molecular techniques. The serological techniques include conventional serology and the enzyme linked immunosorbent assay (ELISA) where molecular techniques are biochemical and molecular means of studies. The International Commission on Trichinellosis (ICT) has been recommending since 1993 that the *Trichinella* isolate has to be characterized by genetic means rather than by other methods (Lichtenfels *et al.,* 1994).

2.5.1 Direct demonstration of the parasite in tissue samples and digests

ICT (http://monsite.wanadoo.fr/intcomtrichinellosis/) has recommended that no serological method should be allowed for the testing of individual carcasses of food animals at slaughter. It has also recommended that meat inspected directly through trichinoscopy should only be allowed as a transitional measure and such meat should be clearly marked. Furthermore, such meat has to be limited for the selling on the national market. It is also clearly unacceptable for products where the production process does not kill *Trichinella*. Previously, in the EU legislation (Directive 77/96/EEC) for the direct demonstration of parasites, seven methods were accepted: six digestion methods and trichinoscopy. But in the present EU legislation (SANCO/1900/2002 Rev. 8 draft, in forced 01-2006) the number of inspection methods are reduced to four with the magnetic stirrer digestion as the reference method (Webster *et al.,* 2006), as outlined in table 1.

Table 1: The use of different methods for the direct demonstration of the *Trichinella* larvae in pork and horse meat

Method	Description of technique
Trichinoscopy/Compressorium	It is a rapid method for only few samples. It is laborious and has low sensitivity (detection limit 3-5 lpg). This method does not detect the non-encapsulating *Trichinella*. The sensitivity is less than 43.4% in low larval burden (Beck *et al.*, 2005).
Stomacher (mechanical treatment) and sedimentation or filtration	It needs only a short duration (25 min) but the larvae found adhere to plastic bags. It has lower sensitivity than stated in the EU Directive (1-3 lpg). It gives more false negative results. 1 g and 5 g meat of pig/wild boar and horse, respectively, need to be examined according to this method.
Trichomatic 35 blender	In this method 35 samples can be pooled at the same time. It is easy to manage and needs a very short digestion time (5-8 min). The filter, however, requires extra washing procedures, which is a main demerit of the technique (van Knappen *et al.*, 1996). The detection limit is 1-3 lpg.
Magnetic stirrer (constant mechanical treatment)	It needs a short duration time (30 min/100 g). But the filter size needs adjustment for correct sensitivity of 1-3 lpg. The recovery of the larval improves if the filter size changes from 177 μm to 355 μm (Gamble, 1999). 1 g and 5 g of meat need to be examined according to the EU directive.

2.5.2 Indirect demonstration of parasites through serological and molecular techniques

Sero-diagnostic methods are used for the ante mortem as well as for the post mortem examination of the serum samples for *Trichinella* specific antibodies. Under some conditions these techniques may have a higher sensitivity than that of direct detection (Nöckler *et al.,* 2000). It is a useful technique for herd surveillance, for establishing *Trichinella* free areas and for reducing restrictions in international animal trade (SCVPH, 2001). However, the serology will fail to detect both early (<3-4 weeks) infection (Gamble, 1996; Kapel, 2001) and the chronic (>22-23 weeks) infection (Oivanen, 2005).

The serological response in horse is different than in pig (Voigt *et al.,* 1997). In horse the antibody declines within a few months following the infection. This coincides with a decline in the muscle larvae. It makes the serological evaluation of *Trichinella* in horses not significant since even animal harboring hundreds lpg can show a negative serology (OIE, 2004). Another alternative matrix is the use of meat juice in ELISA for detecting *Trichinella* antibodies in animals like previously slaughtered pigs, hunted animals, dead animals etc. which cannot provide blood sera any more. In these days this has increased in popularity (Kapel *et al.,* 1998; Nöckler *et al.,* 2004; Möller *et al.,* 2005).

2.5.2.1 Serological methods

There are several serological diagnostic techniques like the western blot (WB), the complement fixation test (CFT), the haemagglutination test (HAT), the immunofluorescence antibody test (IFAT), the enzyme immunohistochemical technique (EIH) etc. So while choosing any technique the greater attention has to be paid to the technique that has a high validation and quality assurance (Gamble *et al.,* 2004). IFAT and HAT have achieved a higher degree of sensitivity than CFT (Nöckler *et al.,* 2000). However, the many reference laboratories use the enzyme linked immunosorbent assay (ELISA) as a method of choice (Nöckler *et al.,* 2000).

Enzyme linked immunosorbent assay (ELISA)

ELISA is the most common used method for detecting the *Trichinella* infection because it is economical, reliable, readily standardized and provides an acceptable balance of sensitivity and specificity. The ELISA test, using the excretory-secretory (ES) larval antigen or the tyvelose antigen, is useful for the surveillance systems. Several studies have demonstrated that both ES and tyvelose antigens reduce cross-reactions which, however, occur if a crude *Trichinella* antigen is used (Gamble *et al.,* 1997). However, the quality of the ES larval antigen depends on the adherence to proper methods for the cultivation of muscle larvae and the purification of its antigen (Gamble *et al.,* 1988). In pigs the sensitivity and specificity of ELISA using the ES antigen has been reported to range from 93.1-99.2% and from 90.6-99.4% respectively. That means that sufficient time of the infection elapses for the infected animals to develop an antibody response (van der Leck *et al.,* 1992). An Infection as few as 1 lpg of tissue can be detected by ELISA (Kapel and Gamble, 2000). A synthetic glycan antigen, which has a high sensitivity and specificity, is also used for the serological testing (Gamble *et al.,* 1997). The recent application of ELISA in the *Trichinella* diagnostic is the immunochromatographic strip (Zhang *et al.,* 2006) for the rapid detection of analytes. It is easy to use and provides a visual end-point. In the strip the ES larval antigen is labeled with colloidal gold as a detector. The Staphylococcal Protein A and goat anti-ES-antibody are blotted on the nitrocellulose membrane for test and control lines, respectively.

Western blot (WB)

The western blot technique can also be applied to detect antibodies in a serum or to define antigens with the aid of immune sera. With this technique the antigens are recognized by their molecular weight (Robert *et al*., 1996). Industrially produced western blot strips, which seem to work without major cross-reactions, are available for human diagnostics (Yera *et al*., 2003). However, differences exist in western blot patterns observed between human and animal sera infected with the same *Trichinella*

species (Dupouy-Camet *et al*., 1988). Therefore caution should be taken when extrapolating methods to animal diagnostics.

2.5.2.2 Molecular techniques

Biochemical differentiation

The biochemical differentiation of the *Trichinella* genotype via the isoenzyme analysis with starch gel electrophoresis is a very powerful tool for genetic studies at the level of the species and the population. It is possible to infer variability at the DNA level among parasite genotypes via electrophoretic mobility of the enzymatic proteins as markers (Zarlenga and La Rosa, 2000). This technique is able to analyze diverse portions of the genome that have evolved independently of each other. Curran *et al.* (1985) exploit firstly the repetitive and polymorphic nature of the genomes within this parasite group for differentiation. The fluorescence staining is sufficient to demonstrate differences in the organization of the *Trichinella spiralis* and *Trichinella pseudospiralis* genome, simply through digesting the genomic DNA with the restriction enzyme and then separating the segment by the use of agarose gel (Zarlenga and La Rosa, 2000).

Dame *et al.* (1987) were the first to use the restriction fragment length polymorphism (RFLP) as a tool for the investigation of the epidemiology and dissemination of *Trichinella* genotypes in sylvatic hosts. The RFLP technique provides large numbers of bands on agarose gel representing genetic heterogeneity within the genus (Zarlenga and La Rosa, 2000). Chamber *et al.* (1986) have used the DNA probe technology by radio labeling and generated characteristic fingerprints using a genus specific probe TsR1. This technique is used for screening southern blots of the restriction enzyme digested DNA from the same group of parasites. Amplified fragment length polymorphism (AFLP) is a DNA fingerprinting technique. It shows high-resolution power with *Trichinella nativa* (with species specific bands) and is a promising method for detecting population variation (Mikkonen *et al.*, 2005).

All those tests have been used, successfully, to differentiate various *Trichinella* genotypes. They are, however, are either labor intensive, require large sample sizes or use hazardous materials (radioisotopes) and need to work at more sensitive level (Zarlenga *et al.,* 1999). This has led to seek alternative approaches like the adaptation of PCR technology.

Polymerase chain reaction (PCR)

In general, the PCR methods can be divided into those involving random or arbitrary primers and those involving DNA sequence derived genotype specific primers. Welsh and Mc Clelland (1990) and Williams *et al.* (1990) have described an approach using PCR amplification to generate genetic markers from genomes in the absence of any specific sequence information. This method requires a single, randomly designed primer usually 10 base pair in length. It is used under non-specific amplification conditions to generate a population of amplification products in an agarose gel-banding pattern, which is characteristic of a genotype. The fingerprint pattern obtained with this method is randomly amplified polymorphic DNA (RAPD) and this is supposed to be a simple and effective tool for quickly generating and detecting genetic markers (Bandi *et al.*, 1993). It can be demonstrated at a larger set of *Trichinella* species with a single primer by combining RAPD and DNA hybridization (Chacon *et al*, 1994; Dupouy-Camet *et al.*, 1994).

The strengths of the RAPD-PCR are a quick result with high sensitivity. The poor reproducibility either from variation in the sample DNA quality or contamination is the most prominent weakness (Zarlenga and La Rosa, 2000). Another weakness is the necessity of re-optimizing when moving from one laboratory to another (Oivanen, 2005). Single strand conformational polymorphism (SSCP) can be used to characterize and differentiate seven distinct genotypes of *Trichinella* (Gasser *et al.,* 1998). SSCP utilizes heat denaturation to induce strand separation in the PCR amplified products which are then subjected to the non-denaturing gel electrophoresis. This technique shows a great promise for defining both inter and intra specific variations in the expansion of segment five (ESV). Several investigations

have been performed using genotype specific primers in association with variations in the length of the ESV sequence to differentiate *Trichinella* genotypes (Zarlenga and Dame, 1992; Arriaga *et al.*, 1995; Zarlenga *et al.*, 1996; Appleyard *et al.*, 1999). However, the unilateral application of SSCP for rapidly delineating genotypes is not significant since radioisotope markers are needed for the final visualization (Zarlenga and La Rosa, 2000).

The Multiplex PCR test can unequivocally distinguish all currently recognized species of *Trichinella* through the amplification of ESV sequences. It is not only used for differentiating species but also as an internal control for the PCR integrity (Zarlenga *et al.*, 1999). The single or two ribosomal DNA fragments are sufficient for characterizing each genotype via the Multiplex PCR technique. Those genotypes are *T. spiralis* (173bp), *T. nelsoni* (155bp, 404bp), *T. nativa* (127bp), *T. britovi* (127bp, 252bp), *T. murrelli* (127bp, 316bp), *T. pseudospiralis* (300bp, 360bp) and *T. papuae* (240bp) fragments (Murrell *et al.*, 2000). The Multiplex PCR is able to perform on individual larva. So it is easy to use for epidemiological investigations where only some few larvae are available (Zarlenga *et al.*, 1999). If the sample, however, stems from the pooled larval DNA, where the possibility of concurrent infection with several species exists, the result may be ambiguous (Zarlenga and Higgins, 2001).

The reverse line blot hybridization (RLBH) is based on variation in the DNA sequence between the 5S ribosomal DNA genes within the genus *Trichinella.* The amplified 5S ribosomal RNA region is analyzed using a cleavage fragment length polymorphism assay (Oivanen, 2005). This method could identify single larvae and it has 10 fold more sensitive than the agarose gel analysis (Rombout *et al.*, 2001). Recently, molecular characterization by inter-simple sequence repeat (ISSR) PCR is described as new molecular technique (Fonseca-Salamanca *et al*., 2006).

2.6 Diagnostic measures in the human

2.6.1 Parasitological diagnosis

For the identification of *Trichinella* parasites in suspected patients during the rheumatoid phase a muscle biopsy must be collected. In the human, deltoid muscle is preferred because it is more accessible (Gould, 1970b). The trichinoscopy is of great use for identifying the parasites at species level (Zarlenga and La Rosa, 2000). It is also useful for sporadic and doubtful cases, for diagnosing and retrospective analysis (Dupouy-Camet *et al.*, 2002). The histopathological examination method is another technique. It has a higher sensitivity than the trichinoscopy at the early stage of the muscle invasion when the larvae are still very small and not easy to differentiate from the muscle fibers (Wranicz *et al.*, 1998). Even if the larvae are not seen by the histopathology the infected muscle cells undergo basophilic changes once newborn larvae will penetrate it (Capo and Despommier, 1996; Kozek, 2005). The artificial digestion is a more sensitive technique than the direct microscopic observation of tissue (Zarlenga and La Rosa, 2000). To get such sensitivity the digestion has to be carried out not before the days 17-21 after the infection. It is because the larvae of *Trichinella* spp. before that period are not resistant to digestion (Despommier, 1986). The complications for the patient after performing the muscular biopsy are the main obstacle to perform direct demonstration of *Trichinella* in humans (Mahannop *et al.*, 1995).

2.6.2 Sero-diagnosis

The objectives of the sero-diagnosis in humans are an early recognizing of the acute infection for prompt treatments and for a retrospective diagnosis to gather the epidemiological information (Ljungström, 1983). Only from the antibody level in serum it cannot correlate with the severity of a clinical course (Bruschi and Murrell, 2002) because the antibody can be detected even after 19 years of its acute phase (Kozar and Kozar, 1968).

In humans the most common serological tests are the indirect haemagglutination, the bentonite flocculation, the indirect immunofluorescence, the counterimmunoelectrophoresis, the latex agglutination and the ELISA, the last is the most sensitive test (Despommier, 1986; Murrell, 1994; Kociecka *et al.,* 2001). In the ELISA method excretory-secretory larval antigens are preferred, particularly in tropical regions where the cross-reactions with other helminthes *Ascaris*, *Trichuris* and *Filariae* could give false positive result (Au *et al.*, 1983).

A recent study among the Spanish people has reported that the capture ELISA using crude *Trichinella spiralis* larva group 1 (cTSL-1) antigen is the most effective method for the sero-diagnosis of human trichinellosis since it has 100% of both sensitivity and specificity (Escalante *et al.,* 2004). SCVPH (2001) has suggested that the combination of two techniques *i.e.* ELISA and IIFT would provide the most reliable results and in case of a rapid (<1 hour) result, the latex agglutination is recommended.

Immunoblotting is considered to be a confirmatory test in some instances (Bruschi and Murrell, 2002). It is useful for follow up studies (Andrews *et al.*, 1995). However, it cannot determine the species of *Trichinella* responsible for infection (Ranque *et al.*, 2000). Western blotting is a useful tool for investigating ELISA and IIFT cross-reactions (De-La-Rosa *et al.*, 1995). However, possible cross-reactions with cases of anisakiasis (Yera *et al.*, 2003) and schistosomiasis (Gamble *et al.*, 2004) are reported.

2.7 Therapy

In animal models benzimidazole is effective against newborn larvae and for young adults that are on either lymphatic, blood vessels or in intestine. Flubendazole has shown to have high efficiency on gastro-intestinal larvae of *Trichinella*, but very little efficacy on muscular larvae in pigs (Marinculic *et al.,* 2001). However, in practice, pharmaceutical treatment is only used with human patients not with animals, since symptoms in animals have not been clinically noticed.

In humans the diagnosis of trichinellosis is often late due to a delayed onset of non-specific symptoms after an infected meal (Nunez *et al.*, 2003). After proper diagnosis the proper use of benzimidazole anthelmintics (thaibendazole, mebendazole and albendazole) and corticosteriods are recommended for trichinellosis (Dupouy-Camet *et al.*, 2002; Schellenberg *et al.*, 2003). These drugs are effective in limiting the severity and duration of the disease. Mebendazole (200 mg/day for 5 days) or albendazole (400 mg/day for 3 days) is considered to be a first line drug and should be given to adults (except pregnant woman) as well as to children (5 mg/kg body weight for 4 days) (Hermanowska-Spakowicz *et al.*, 1993; Kociecka, 1993). Any of these doses have to be administered along with prednisolone (30-60 mg/day) in multiple doses for 10-14 days (Dupouy-Camet *et al.*, 2002).

Pyrantel (10 mg/kg for 1-3 days) has been used to treat pregnant woman and young children. This is because pyrantel is not absorbed from the intestinal lumen and acts by paralyzing mainly intestinal parasites (Kociecka, 2000). Mebendazole has shown to prevent occurrence of trichinellosis when given to people 48 hours after consumption of meat which was heavily infected with *Trichinella* (Kociecka *et al.*, 1996). But if the muscle larvae are already encapsulated then the mebendazole is not sufficient to control the infection (Pozio *et al.*, 2001c). If the infection is due to non-encapsulated species then the albendazole (800 mg/day in 4 doses) is particularly efficient (Jonwutiwes *et al.*, 1998). Emodepside (cyclo-octa-depsi-peptides) is fully effective against benzimidazole resistant nematodes and was found to be most active against *Trichinella spiralis* (Harder *et al.*, 2003).

2.8 The control of *Trichinella* in domestic animals

The existing control methods of *Trichinella* are not infallible. So uniform control measures at farms, slaughters and in the processed meat level are needed (van Knapen, 2000). Since there is such a need the recommendations provided by the International Commission on Trichinellosis (ICT) are based on the best scientific information available (Gamble *et al.*, 2000).

The controls at the farm level include requirements for *Trichinella* free pig production through architectural and environmental barriers, proper feed and feed storage, rodent control and farm hygiene. In addition to that good management practices at the farm and periodic serological testing are also necessary (Murrel and Pozio, 2000). The animals that have access to the environment and/or to potentially contaminated feed (swill, carcass etc.) will always constitute a potential public health threat with regard to trichinellosis. Consequently, such animals must undergo proper inspection at the individual level before they are declared to be suitable for human consumption (van Knapen, 2000). In the US, a pilot program for certifying a pig herd was conducted (Pyburn *et al.,* 2005). However, candidate vaccine through utilizing antigens of the newborn larval was developed (Marti *et al.,* 1987). This approach to eradicate *Trichinella* in herds where the management strategies had not been completely successful is not posing real value. This is also because the costs of the production and application are unacceptably high (Murrel and Pozio, 2000).

Slaughter inspection methods are designed to prevent clinical trichinellosis in the human (Gamble *et al.,* 2000). Therefore carcass infected with *Trichinella* should be condemned (Herenda *et al.,* 2000). The pooled digestion method, using a minimum of 1 g of sample is considered to be sufficient for detecting parasites at the slaughterhouse level. The development and use of proper quality assurance systems including training and education of personnel, test validation and control of critical control points are the prerequisites for minimizing the risks at the slaughterhouse level (Forbes and Gajadhar, 1999).

ICT has recommended that meat from animals that might contain *Trichinella* and are not tested by acceptable methods must be treated with a processing (cooking, freezing and eradication) method. The processing methods have proven to inactivate *Trichinella* for human consumption (Gamble *et al.,* 2000). Freezing meat below the critical temperature of -30^0C will kill *Trichinella* larvae in an appreciable period of time (Smith, 1975). It depends on the thickness of the muscle cut. 15 cm thick muscle cuts need to be kept for 3 weeks and 69 cm for 4 weeks at ≤ minus15^0C (Gamble *et al.,* 2000). ICT has also recommended cooking at an internal temperature of 71^0C

(160^0F) for totally safe consumption. The change in the meat color from pink to grey and likewise the change in texture (muscle fibers are easily separated from each other) are the indicators which show that the meat has been rendered and safe for the consumption (Gamble *et al.*, 2000). The methods of preparation of meat in the microwave cooking, curing, drying, smoking etc. are not considered to be secure (Gamble *et al.*, 2000).

The motile larvae of *Trichinella nativa* was found in the muscle tissue of a fox which was frozen for 1 year at -18^0C (Kapel *et al.*, 1999). The study conducted by Worley *et al*. (1986) in frozen skeletal musculature of wild carnivores has also revealed that 50-60% of the larvae in grizzly bear meat are alive even after having been stored for 27 months at -6.5^0C to -20^0C. This is because the antifreeze protein molecule which is common in most wild animals protects the worms in their muscle tissue from getting ice crystallized (Dick, 1983).

2.9 Economic impacts

As a result of the mandatory inspection the European Union spends, annually, US$ 570 million for controlling *Trichinella* infection from slaughtered pigs (Pozio, 1998), and around US$ 5 million a year for controlling the infection from horse carcasses (Pozio, 2000a). The annual economic cost of swine and human trichinellosis in the United States has reached over US$ 1 billion for health regulatory activities to prevent the infection which includes the utility costs for treating pork through freezing/heating/curing (Roberts and Murrell, 1993; Roberts *et al.*, 1994; Bruschi and Murrell, 1999). The cost for each human infection is estimated to amount around US$ 6000 (Robert *et al.*, 1994) in the United States and around US$ 3000 in France (Pozio, 2000a). Such direct and indirect costs have turned trichinellosis into being one of the most costly parasitic zoonosis (Murrell and Pozio, 2000). One example for the economic loss is a Chinese abattoir which (due to high prevalence of *Trichinella* infection in the slaughtered pigs) lost in-between 1975-1985 US$ 550 000 (Wu, 1995).

2.10 Trichinellosis in animals and humans in the global context

Over the past decades trichinellosis has been recognized in many parts of the world, in new hosts and with new epidemiological contexts, except from the desert zones (Pozio *et al.*, 2002). From a public health point of view the situation is worrisome in Argentina, Croatia, Yugoslavia, Russia, Romania, Latvia, Lithuania and China. The most reported sources of the origin of *Trichinella* infection in human being are from pork and horse meat. The other unusual meat of animals are also potential sources for the outbreak of the disease in humans such as bears in Greenland, Canada, USA, Japan, Eastern Europe and China (Soule, 1991), walrus in Canada and Alaska (Morgolis *et al.*, 1979), cougar in USA (Dworkin *et al*, 1996), fox in Italy (Dupouy-Camet, 2000), mutton in China (Wang *et al.*, 1998), warthog in Africa (Kefenie and Bero, 1992) and dog in Slovakia and Thailand (Dubinsky *et al*, 1999; Srikitjakarn *et al.*, 1981). Irrespective of that Wang *et al.* (2007) has documented *Trichinella* infection in cattle, muntjak, bamboo rat, weasel, shrew and also in moles. Recently, *Trichinella* infection in farmed fur animals was also reported in Estonia (Miller *et al.*, 2006).

In the USA, pigs are not tested, regularly, for *Trichinella* parasites. In a survey of 180 (n = 4078 pigs) farms in the northeastern states 0.37% prevalence and 6.4% herd prevalence were reported (Gamble *et al.*, 1999). The most common source of infections is pork but infections from wild game are also increasing from 27% to 42% (Moorhead *et al.*, 1999). The number of human cases with trichinellosis, however, has been decreasing since 1947. In-between 1991- 1995, 230 human cases were reported and out of those 3 patients have died (Oivanen, 2005).

In Canada, *Trichinella* infection in pigs was reported only in the Nova Scotia region and sporadically in farmed wild boars. A national serologic survey (1996/97) in pigs with using ELISA technique has shown no positive result (Gajadhar *et al.*, 1997; Appleyard *et al.*, 2002). Human outbreaks have occurred due to the consumption of wild game and raw walrus meat (Dick *et al.*, 1986; Schellenberg *et al.*, 2003). In-between 1991-1997 about 3 to 49 human cases, annually, were reported,

mainly from the northwest-territories and from the Quebec region (Murrel and Pozio, 2000).

Trichinellosis is still endemic in most countries of the EU. Denmark and The Netherlands are free from domestic trichinellosis but sylvatic trichinellosis in low prevalence is still reported in these countries (Pozio, 1998). According to the annual reports of zoonotic agents in the EU, domestic trichinellosis was detected in Italy, France, Finland and Spain, whereas sylvatic trichinellosis was found in Austria, France, Finland, Germany, Ireland, Italy, the Netherlands, Spain and Sweden in-between 1996-2003. At the British Isles, however, there has been no report of this parasite. Recently, *T. britovi* infection in a sow was reported from Sardinia and Corsica which were previously seen as *Trichinella* free Mediterranean islands (Pozio *et al.*, 2006).

In Germany the domestic pig and horse population are considered to be free from *Trichinella* (Nöckler *et al.*, 2006). However in-between 1991-2003 about 156 wild boars were found being infected. Concerning the species in polar bears *T. nativa,* in wild boars and raccoon dogs *T. spiralis* and in red foxes both *T. spiralis* and *T. britovi* were detected (ITRC, 2005; Pozio *et al.*, 2000). The mixed *T. spiralis* and *T. pseudospiralis* infection was detected in a wild boar from Mecklenburg, Germany (Nöckler *et al.*, 2006).

Outbreaks of trichinellosis appear to increase in the EU member states. But this trend is difficult to appreciate. Pozio (2000b) has calculated over 3300 human cases of trichinellosis in France and Italy (1975-2000) from the consumption of imported horse meat. No human trichinellosis from the consumption of local domestic or wild animals (except from Sweden, where about 2600 cases were reported at that time) was reported from Austria, Belgium, Britain, Denmark, Finland, Ireland, Luxembourg, the Netherlands and Portugal in-between 1978-1998 (Pozio, 1998). However, in 1999 the Netherlands, Germany, Spain, France, the UK and Austria have reported all together 49 human trichinellosis cases with an increasing tendency of 88 cases in the year 2000 (SCVPH, 2001). The total human infection, acquired in the EU in-between 1999-2003, ranged from 48 to 67 per year. Recently in 2007, an outbreak

of 21 cases of *Trichinella britovi* infection has reported among persons of Spain and Sweden related to Spanish wild boar sausage (http://www.promedmail.org).

Human infections were reported, annually, in all of the new EU member states Bulgaria, Byelorussia, Poland, Romania, Serbia and the Ukraine (Pozio, 2001a). In Romania the incidence of infection have increased 17 times since 1983 (Olteanu, 1997). In 2007, an outbreak of trichinellosis in Zachodniopomorskie voivodeship (West Pomerania) Poland has affected 201 people (Golab *et al*., 2007). Later on 4 imported cases from victim of that outbreak are also reported in Hamburg, Germany (http://www.promedmail.org). In Eastern Europe, Slovakia and Kazakhstan there is an emergence of non-pork sources like dogs, wild games and wild boars as source of infection (Murrell and Pozio, 2000; Pozio, 2001a). In the Tvier and Smolensk region of Russia the prevalence of *Trichinella* in wolf, fox and domestic dog is as high as 97.3%, 48% and 7.7%, respectively. The carcasses of these animals seem to maintain the infection in the population (Casulli *et al.*, 2001).

Trichinellosis is also a major public health problem in South America. In Mexico, *Trichinella* infections are reported in pig, rat, cat and dog in several states. About 758 human trichinellosis cases were found in between 1952-1997 (Ortega-pierres *et al*., 2000). In Argentina during 1974-1983, 894 human cases were reported from 15 provinces (Ortega-pierres *et al.*, 2000). During 1990-1999 an unexpectedly high number of 5217 cases was documented (Murrell and Pozio, 2000; Bolpe and Bofi, 2001). In Chile at least 26 outbreaks (where 1300 peoples were infected) happened in between 1981-1995 (Ortega-pierres *et al.*, 2000).

In Sub-Saharian Africa the sylvatic trichinellosis was confined to wildlife living in natural parks and protected areas where the spotted hyena is the main reservoir with a prevalence of 43-85% (Pozio *et al.*, 1997). Trichinellosis in human is derived from the sylvatic cycle with ≤100 human infections reported from Ethiopia, Kenya, Senegal and Tanzania. The low level of the infection in sylvatic suidae relates to the practice of eating only cooked meat and the religious laws that forbid pork consumption (Murrell and Pozio, 2000).

In Australian region, *Trichinella pseudospiralis* is widespread in the Tasmanian wildlife (Obendorf *et al.,* 1990; Obendorf and Clarke, 1992). In Papua New Guinea *T. papuae* was identified in domestic and sylvatic swine (Pozio *et al,* 1999a). The first human case of *T. pseudospiralis* infection was noticed in New Zealand (Ainsworth *et al*., 1994).

Trichinella found in Asia and the Pacific Rim includes both encapsulated species and non-encapsulated species. It is assumed that numerous outbreaks of trichinellosis have happened in these regions but insufficient epidemiological data are available for many countries (Takahashi *et al*., 2000). In Japan the first case in raccoon dogs was reported in 1957 and after that several investigators have isolated *Trichinella* from black bears that were identified as T9 (Nagano *et al.,* 1999). It is likely that trichinellosis has occurred at a low level in Japan for many years but the outbreak of 1980 that has infected 60 peoples has increased the intention of Japanese physicians to this disease (Takahashi *et al.,* 2000). In Central Asia *Trichinella* (*T. nativa, T. britovi)* in wolf, red fox and golden jackal was documented (Shaikenov and Boev, 1983).

In Southeast Asia *T. spiralis, T. pseudospiralis* and *T. papuae* were detected in sylvatic and domestic animals and in humans (Pozio, 2001b). Trichinellosis has occurred in all parts of Thailand but is more abundant in the northern areas where 11.4% of hill tribe pigs and 0.02% of the commercial pigs are positive (Takahashi *et al.,* 2000). The source of infection has acquired through consumption of local dishes called lahb and nham. The source of human infection in the country stems mainly from the hill tribe pigs', jackals', black bears' and rats' meat (Suriyanon and Khunklin, 1972). An examination of a dog's diaphragm in the Sakon Nakorn province where dog meat is used for human consumption has revealed that 1.6% (n = 421) of the dogs were infected with trichinellosis (Srikitjakarn *et al.,* 1981). It was reported that in-between 1962-1991 there were about 118 outbreaks in Thailand which infected 5400 people and out of that 95 have died (Khamboonruang, 1991). In Indonesia, Laos, Malaysia and Myanmar, the *Trichinella* infection was also reported in domestic animals and/or humans, referred by Pozio *et al.*(2001b). A sero-prevalence of 1.4% was reported in Cambodia (Sovyra, 2005). Chomel *et al*. (1993) have reported a high

sero-positive rate (19.5%) of trichinellosis among children and teenagers in Bali, Indonesia. Recently, an outbreak investigation has confirmed 22 trichinellosis cases through western blot in Laos (Sayasone *et al.*, 2006).

In China, the greatest spreading of the infection was documented from Hubai, Henan, Yunnan, and the Guangxi provinces with two epidemiological cycles, *Trichinella spiralis* in pigs and *Trichinella nativa* in dogs (Liu and Boireau, 2002). In these provinces 5% prevalence were reported in pigs (Cui *et al.*, 2006) whereas in the northern, southern and coastal regions 7.5% prevalence were found (Chan and Ko, 1992). The prevalence of *Trichinella* is 1.2% (2/163) in cattle, 1.4% (3/215) in sheep, 2.1% (1/47) in beef cattle and 16.2% (5654/34983) in dog (Wang *et al.*, 2007). This infection in herbivorous is probably associated with ingesting either hay or forage mixed with raw swill containing pork scraps or grazing in contaminated pastures with infected rodent carcasses. The highest number of human cases (with a mortality of 0.95%) was reported in China (Takahashi *et al.*, 2000). In-between 1964-1999 there were 548 outbreaks of human Trichinellosis. 525 of them were associated with eating pork and the remaining 23 outbreaks were related to the consumption of goat, dog and game meat (Wang *et al.*, 2007). In that period 25 161 cases were reported with 240 deaths (Lui and Boireau, 2002). The occurrences of human trichinellosis (1992-1996) in the Henan province have shown that the peak of the infection rate is found in winter; a decrease in spring and a lower number in summer and autumn (Wang *et al.*, 1998). In-between 2000-2003 there were 17 outbreaks of human trichinellosis with 828 cases and 11 deaths reported (Wang *et al.*, 2006).

For the Indian subcontinent there are only some very few old reports that describe the presence of *Trichinella* in synanthropic and sylvatic animals (Murrell and Pozio, 2000; Mohan *et al.*, 2002). The first human case in India has been reported, recently (Mohan *et al.*, 2002; Handa, 2003). In Nepal, however, no human case has been documented yet. A study conducted in Hong Kong on the sera of 18 Nepalese soldiers with clinical manifestations of acute trichinellosis has found 94% positive from IHA, RIA and ELISA and 56% positive by muscle biopsies. That has revealed the presence of the infection among the population (Au *et al.*, 1983).

2.11 Veterinary public health regulatory measures on trichinellosis control

Trichinella inspection for every slaughtered pig is mandatory in many of the EU member countries whereas in some countries it is required only for pork trading to other member countries (Nöckler *et al.*, 2000). The EU is currently preparing a legislation to set aside the testing on *Trichinella* free farms and/or areas. A similar intent has also emerged in North America (Oivannen, 2005). In the USA meat inspection for *Trichinella* is not mandatory. Other methods for controlling infections in animals and humans are used like the consumers are advised to cook or freeze pork properly at home (Bruschi and Murrell, 2002) and good farm management practices including rodent control and avoidance of feeding waste to pigs are implemented at the farm level (Gajadhar and Gamble, 2000). However, the increasing interest in bio-organic pig farming may bring drawbacks and such an interest needs to be tackled with new aspects of control for ceasing *Trichinella* infection (Pozio, 2001a; Oivannen, 2005).

The quality assurance requirements in the laboratory analysis will eventually impact *Trichinella* diagnostics at meat inspection worldwide. The methods that have used in each laboratory must be validated (Gamble *et al.*, 2000), so that the test results generated from the laboratory are reliable and scientifically defensible according to the defined parameters of the tests (Gajadhar and Forbes, 2002). In Canada and in some other countries, an obligatory proficiency testing of laboratories is already in place (Gajadhar and Forbes, 2002; Pozio and Christensson, 2004). In the areas where irregular outbreaks of trichinellosis are reported either as small family outbreaks or large-scale urban type outbreaks it is obvious that continued meat inspection at the abattoir is necessary (van Knapen, 2000). In such endemic areas an individual carcass should be examined by a method with high sensitivity (Pepsin digestion method) but not with serological testing (van Knapen, 2000).

A decade ago the Chinese food hygiene regulation (FHR) had tolerated that carcasses with light infection could be consumed after high temperature treatment (Lui and Boireau, 2002). After 1996 a new FHR has implemented. It is recommended that the infected carcass must be destroyed even if there is only one larva found in

carcass. To increase sensitivity the pooled sample digestion technique was implemented in slaughterhouses (Lui and Boireau, 2002). In Nepal there is a lack of reliable epidemiological information on zoonotic diseases of public health importance (Gongal, 2003). However, in 2001 the slaughterhouse and meat inspection regulation was passed by the government (FAOLEX, 2001) but it has not been implemented yet (Gongal, 2003). There is an urgent need for harmonizing the national food safety standard with the international standards and a sanitary phyto-sanitary measure.

In addition to improved control methods there is a need to accurately record and communicate information regarding the occurrence of *Trichinella* in man and animals (Kim, 1991; Dupouy-Camet, 2000). The computerized database for recording animal and human occurrences is integral for assessing the risk of trichinellosis in food animals from around the world (Polley *et al.*, 2000). To accomplish control in all parts of the world and to overcome problems associated with global commerce it is necessary for international organizations like ITC, OIE, FAO to play key roles in standardizing, accrediting and ensuring equivalency of protocols for the global control of *Trichinella* infection (Gajadhar and Gamble, 2000).