INTRODUCTION

Background

The genus *Trichinella* has the widest geographical distribution and the largest range of host species (Wakelin and Goyal, 1996). *Trichinella spiralis* can establish in warm blooded animals with the exception of birds while *Trichinella pseudospiralis* can establish in both birds and warm blooded animals (Pozio, 2001). *Trichinella zimbabwensis* has been reported to survive in a wide range of warm and cold-blooded vertebrates (Pozio *et al*., 2004)

Several factors have been implicated in the influence of habitat selection, establishment of a suitable microenvironment and survival of *Trichinella species* in the host (Mettrick and Podesa*,* 1974; Novak *et al*., 1993; Urban *et al*., 2000; Mukaratirwa *et al*., 2003; Gounaris, 2002; Gounaris *et al*., 2004). Among these factors pH, osmolality and biochemical gradient are suspected to play a role.

Hyper-secretion of gastric acid induced by insulin in a dose dependant manner was responsible for the posterior migration of *Hymenolepis diminuta* (Cho, 1984). Raising the gastric pH has been reported to significantly increase the establishment and fecundity of *T. spiralis* adult worm count in both *in vivo* and *in vitro* studies (el-Temsahy, 2001). On the other hand, a decrease in gastric pH induced prior to *Trichinella* infection led to the amelioration of the parasite (el-Temsahy, 2001). Tortora and Grabowski (2003) reported chemical reactions that take place in the body to be very sensitive even to small changes of pH of the body fluids in which they occur. Dinning *et al*. (1999) suggested that rapid changes in caecal pH and osmolality are likely to accompany bursts of ileal

outflow, and changes in one or both have been shown to induce colonic propagating pressure waves (Hardcastle and Mann, 1970).

The absorption and secretion of ions (Na^+, K^+, Cl^- and HCO_3^-) and metabolites is necessary for normal body fluid homeostasis in all vertebrates (Ganong, 2001; Tortora and Grabowski, 2003). These ions and metabolites must be transported across layers of epithelia in the intestines. Of the entire intestinal fluid load, 25% arises from oral intake and the rest from endogenous secretion in the gastrointestinal tract (Tortora and Grabowski, 2003). Fluid secretion into the lumen maintains the fluidity of intestinal contents. Eight five percent (85%) of the fluid is absorbed before reaching the colon, illustrating the important function of the small intestines in both absorptive and secretory processes.

Ladas *et al*. (1983) reported the composition of upper jejunal contents after a meal which reflected that of the gastric contents and despite mixing with upper gastrointestinal secretions and transepithelial movement of fluid and electrolytes, osmotic and electrolytic equilibration of intestinal contents with plasma was not produced up to 2 hour following a meal (Ladas *et al*., 1983). The change in osmolality from the duodenum to the jejunum is normally attributable to increased electrolyte concentration, carbohydrate digestion and net water flux (Gisolfi *et al*., 1998).

Artificial increases in fluid secretion through administration of serotonin reduced the establishment of *T. spiralis* infection in naive rats (Zhang and Castro, 1990). Changes in motility likely combined with other mechanisms, including increased fluid secretion to evict parasites from the gut render the intestine an inhospitable environment for the

parasite, leading to a decrease in fecundity and expulsion of the parasite (Wakelin, 1993; Urban *et al*., 1995; Collins, 1996).

Hypothesis

The hypothesis in this study is that physiological factor changes occur in the establishment of *Trichinella zimbabwensis* in the small intestine of rats and chickens.

Objectives

The main objective of this study was to determine the intestinal pH and osmolality levels of rats and chickens in the presence of adult *T. zimbabwensis*.

The specific objectives were:

1. measure the pH and osmolality levels in the small intestine of rats (*Rattus norvegicus*) infected with *T. zimbabwensis*.

2. measure pH and osmolality levels in the small intestine of chickens (*Gallus gallus)* infected with *T. zimbabwensis*.

3. measure the length of adult male and female *T. zimbabwensis* in the anterior and posterior portion of the small intestine as an indicator of possible investigation of the role played by biochemical gradients.

LITERATURE REVIEW

Trichinella infects a wide spectrum of hosts including humans, mammals, birds, reptiles, varans, caimans, fish, crocodiles, herbivores, ostriches, carnivores and omnivores (Bessonov, 1992; Reina *et al*., 1996; Mukaratirwa and Foggin, 1999; Asatrian *et al*., 2000; Theodoropoulos *et al*., 2000; Piergilli Fioretti *et al*., 2001; Pozio *et al*., 2004).

Trichinella and its hosts

Trichinellosis is an important food-borne zoonosis because of its epizootic nature and the economic burden associated with preventing its incursion into the human food chain (Murrell and Pozio, 2000). Its increasing importance even in developed countries is exemplified by the fact that over 20 000 cases occurred in Europe from 1991-2000 (Bruschi and Murrell, 2002). The role of game animals as a source of infection for humans has greatly increased both in developed and developing countries such as Canada, USA, Bulgaria, Lithuania, Zimbabwe, Papua New Guinea and EU countries (Mukaratirwa and Foggin, 1999; Pozio, 2001).

Prevalence of trichinellosis in animals and humans has been linked to an increase in the number of small farms in countries such as Argentina, China, Mexico and others (Pozio, 2001).

The sylvatic cycle has been studied in depth at both the epidemiological and biological level, showing the existence of different etiological agents namely *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni* in different regions. Recent reports also indicate that infected herbivores (horses, sheep, goats, and cattle) have been the source of outbreaks, a

new variation on the traditional model of trichinellosis epidemiology (Bruschi and Murrell, 2002).

The new emerging patterns are related to non-encapsulated species of *Trichinella* (*T. pseudospiralis, T. papuae, T. zimbabwensis and T. sp*) and encapsulated species (*T. spiralis*, *T. britovi and T. murrelli*) found infecting a wide spectrum of hosts (Pozio, 2001).

The existence of non-encapsulated species infecting birds had remained unknown because of the difficulties in detecting larvae in muscle tissues and for the lack of knowledge on the role of birds as reservoir of *Trichinella* (Pozio, 2001). Most *Trichinella species* fail to establish themselves in chickens with the exception of *T. pseudospiralis*. Ostriches are reported to have a low susceptibility to *T. pseudospiralis* with muscle tissues of the legs being the preferential sites of larval distribution while *T. spiralis* is only found in the bird's muscle tissues when a high number of larvae (80 000) is inoculated (Piergilli Fioretti *et al*., 2001).

Unlike the other *Trichinella spp, T. zimbabwensis* has been reported to survive in a wide range of cold-blooded vertebrates with one of the longest survival duration of adult *T. zimbabwensis* parasites reported to be more than 60 days especially in fish (F Mukaratirwa and Foggin, 1999; Mukaratirwa personal communication; Pozio *et al*., 2004).

Location and distribution of adult *Trichinella*

The intestinal tract serves as a habitat for several parasitic nematodes of considerable significance to human and animal health. The intestinal habitats occupied and niches established by these different organisms vary considerably. The distribution of

Trichinella species in the host like that of other helminth parasites is not random as they occupy specific habitats (Kennedy, 1984; Holmes, 1973; Sukhdeo, 1991). Within the host animal, majority of adult *T. spiralis and T. zimbabwensis* have been reported to inhabit the anterior part of the small intestines in mature rats (Larsh and Hendricks, 1949) and in (young and old) mice and hamsters (Larsh and Hendricks, 1949; Belosevic and Dick, 1979; McCracken, 1982; Mukaratirwa *et al*., 2003). There are also contrary reports on where the majority of adult worms of *Trichinella spiralis* are found inhabiting the posterior part of the small intestine in young rats, mice and guinea pigs of unspecified age (Larsh and Hendricks, 1949). *Trichuris muris* and *T. spiralis* have been reported to share an unusual micro-environmental niche for metazoan pathogens during their enteral phase, being partially or entirely embedded within host enterocytes depending on their life cycle stage.

Sukhdeo and Croll (1981) reported the recovery of the majority adult worms from the site of innoculation, an indication that there is no absolute specificity in habitat selection for the normally selected sites in the intestine but establishment being better in some locations than others with optimum conditions in the anterior small intestine and survival possible in the large intestine.

Parasite feeding mechanisms

All *species* of the genus *Trichinella* have a life cycle in which the parasite completes an entire generation within the body of a single host.

Trichinella spiralis larvae develop normally to the adult stage in the absence of orally ingested nutrients. Castro *et al*. (1976) concluded that exogenous food in the gut is not necessary for maintaining established *H. diminuta* and that factors necessary for

sustainance and growth can be acquired by the worm entirely from the exocrino-enteric circulation. It is speculated that the parasite acquires at least a portion of its nutrients by intercepting metabolites which are carried across the small intestinal epithelium and / or nutrients transported out from the capillaries to the columnar epithelium (Castro *et al*., 1974). With no changes in the sex ratio and worm size, the number of *T. spiralis* recovered from intravenously fed rats was significantly higher than that for the orally fed rats (Castro *et al*., 1976).

In an enteric stage study of *T. spiralis*, it was demonstrated that as embryogenesis begins (48 h p. i), glucose absorption *in vitro* by the parasite rises dramatically to a peak in 72-h-old worms. Absorption of glucose *in vitro* remained high in 96-h-old worms but as larviposition began, glucose intake by *T. spiralis* dropped significantly (Stewart *et al*., 1986).

The excretory/secretory proteinases of adult *T. spiralis* that include serine proteinases are crucial for the establishment and maintenance of the parasite within the host (Todorova *et al*., 1995). Serine proteinases are involved in a broad range of biological processes that include intra and extracellular protein metabolism, digestion, blood coagulation and clot dissolution. Proteinases of this class identified in other parasitic helminth may facilitate evasion of host immune responses in addition to more non-specific roles in parasite nutrition and the penetration of host tissue barriers (Auriault *et al*., 1981; Knox, 1994). It is less obvious why adult *Trichinella* should acquire this activity but it is possible that the parasite feeds on serous exudates resulting from intestinal damage and inflammatory responses initiated by infection with the anti-coagulant activity maintaining the flow of nutrients (Todorova *et al*., 1995).

Trichinella male and female characteristics

Studies using scanning electron microscopy, biological characteristics and molecular techniques have revealed significant distinguishing morphological features of the *Trichinella* isolates (Wakelin and Goyal, 1996 and Kapel *et al*., 1998).

Trichinella spiralis male worms are about 1.4 - 1.6 mm long. They do have terminal anus but no spicules and they have a twin terminal appendages and papillae. Like other members of this order, they have a stichosome, following a short muscular esophagus. The male reproductive system consists of a single tube with a hairpin-like bend, composed of a basal lamina, epithelial cells, rachis, circumferential and constrictor muscles, and germinal cells. The organs are surrounded by basal lamina and haemolymph. Germinal cells in different stages of maturation are found on the wall of the testis along its entire length. As the maturation of germinal cells proceed, the cells move towards the lumen of the testis. The germinal cells have a row of vesicles (cup-shaped structures) at the cell periphery. The mature sperm, lacking flagella and an acrosome, are stored in the seminal vesicle. The cytoplasm of the epithelial cells of the seminal vesicle and ejaculatory duct is filled with distended rough endoplasmic reticulum (rER) and exocrine granules which appeared homogenous and of medium electron density (Takahashi *et al*., 1994).

Trichinella female worms are larviparous and are about twice the length of males with a similarly located anus. The vulva is located about half way along the pharynx, which occupies about 1/3 of the total body length. The single uterus is filled with

developing eggs in its posterior region, but towards the anterior it has fully developed and hatched eggs (Takahashi *et al*., 1994).

Life cycle

The adaptive value of habitat selection behaviors in parasites is still not understood even though literature is full of examples of parasites migrating and navigating through hosts to their specific habitats (Bansemir and Sukhdeo, 1996). Thus parasites must make the same decisions that every animal has to make regarding food acquisition, shelter and reproduction (Bansemir and Sukhdeo, 1996).

Trichinella species can be transmitted to humans through ingestion of under-cooked infected meat and the disease caused by this genus is of great concern from a zoonotic point of view. The larvae remain viable in the muscle of a dead host for a considerable period. Transmission occurs when muscle tissue containing infective larvae is ingested by another animal, human being or bird in case of *T. pseudospiralis*. Ingestion of infected muscle is followed by digestion of the muscle tissue which liberates the larvae from their cysts. Previous studies have shown that *T. spiralis* isolated from host muscle in pepsin-HCl consist of four layers on the surface of infective larvae with pepsin-HCl causing partial degradation and removal of large patches of the two outer surface layers while exposure to bile resulted in, only traces of the outer layers remaining (Despommier *et al*., 1978).

The activation processes of *T. spiralis* infective larvae are stimulated upon liberation of the larvae from the nurse cell inside the host stomach as shown by tracking the activation processes of *T. spiralis* infective larvae through measuring the activities of

the key regulatory enzymes like phosphoenolpyruvate carboxykinase and pyruvate kinase, as well as isocitrate dehydrogenase (Janssen *et al*., 1998).

The complete life cycle occurs in a single host and comprises the adult stage in the gastrointestinal tract and a migratory phase in which infective larvae pass via the blood and lymphatics to skeletal muscle cells followed by developmental arrest and encapsulation until infected muscle is consumed by another host (Todorova *et al*., 1995)

Most *T. spiralis* larvae and adults become localized in the region of the small intestine lying beneath the columnar epithelium and above the laminar propria within 10 minutes of infection with no morphological alterations even though the columnar epithelium would be greatly distorted resulting in the release of high concentrations of nucleotides (Despommier *et al*., 1978; Gounaris, 2002). However transmission electron microscopy studies of damaged cells along the trail of *T. spiralis* showed a progressive increase in size, disruption of cell membranes, loss or dilution of cytoplasmic proteins and swelling of mitochondria and nuclei of the epithelial cell although no nuclear fragmentation were observed (Wakelin *et al*., 1998).

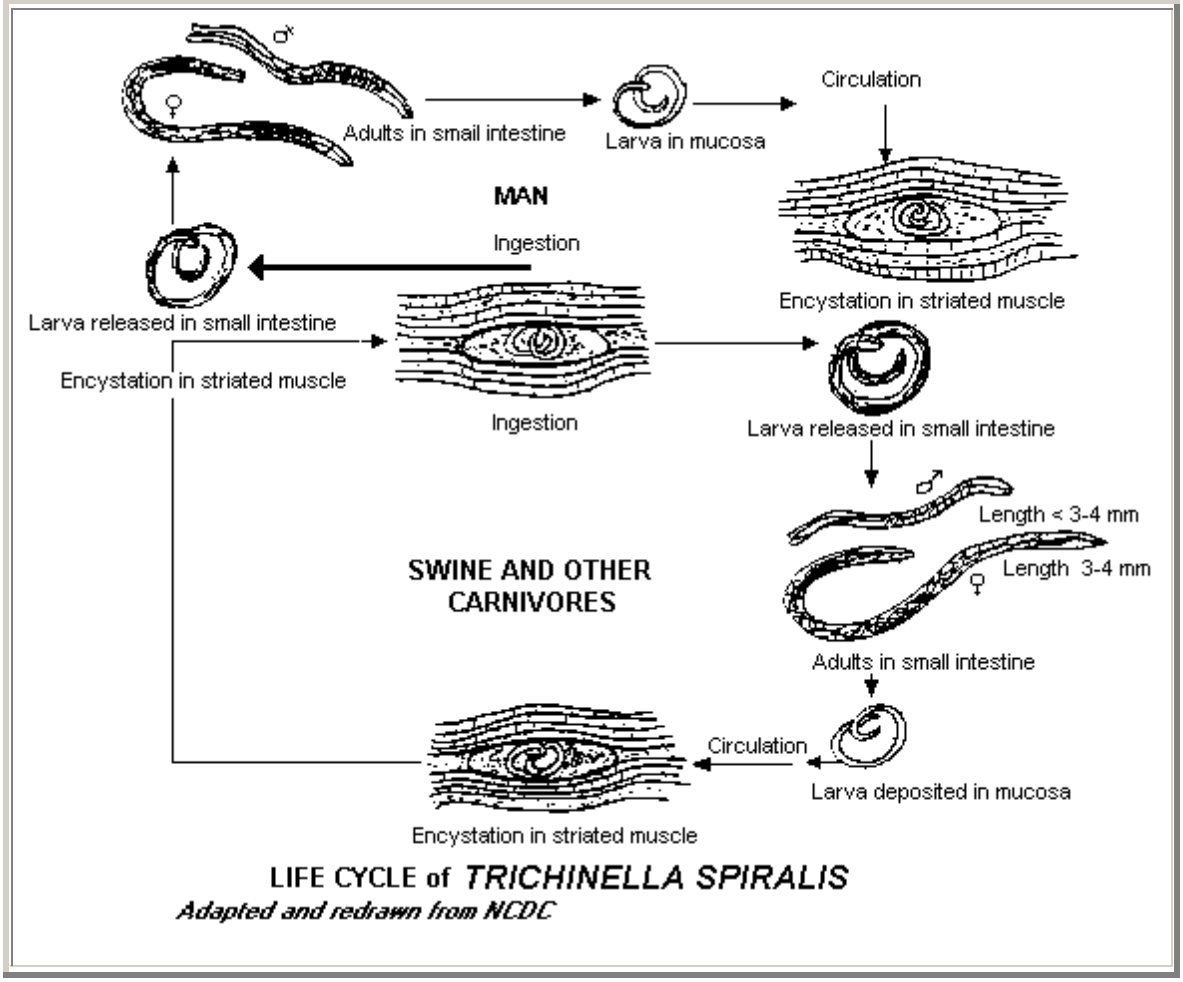
Larvae molt four times in 30 to 40 h following infection (Khan, 1966; Kozek, 1971). The first molt is initiated 8 to 14 h p. i. Worms copulate soon after the final molt and ecdysis (Gardiner, 1976). However *in-vitro* molting and ecdysis require entry of the parasite into cells with conditions that prevent entry into cells also preventing ecdysis (Gagliardo *et al*., 2002). Occupation of cells may stimulate larvae to produce the protease(s) necessary to digest the cephalic end of the sheath. In addition, the cells may provide the physical support necessary for the worm to shed the old cuticle, as suggested

by Despommier (1983). Little growth occurs during the four larval stages of *T. spiralis*, but a period of rapid growth occurs in adult stage females (Khan, 1966).

Following experimental infection of rodents, *T. spiralis* larvae and adults are often found in intestinal epithelial cells at the crypt-villus junction (Gardiner, 1976). The physical organization of this site is quite similar to the physical organization of a monolayer of epithelial cells. Both habitats allow worms to migrate through large number of cells. The density of the worms in the epithelium of the intestine must be very low as the surface area is vast (Gagliardo *et al*., 2002). After mating the males die while the females probably remain in the same niche (Gardiner, 1976). The fertilized eggs hatch inside the uterus of the worm and the larvae are released from day 5 p. i (Urquhart *et al*., 1998). In a primary infection the shedding of larvae by female worms begin on day 5, reaches its peak on days 6-7, begins to decrease on day 8 and is minimal by day 10 (Kennedy, 1980).

The larvae move through the lymphatic system, enter the blood via the thoracic duct and are distributed all over the body especially in slow tonic muscles with a well developed blood supply where they enter into striated muscle cells (Stewart and Chaniga, 1980). Nurse cell cytoplasm in the cyst of *Trichinella* differs among the *Trichinella species* and in the origin of the cytoplasm as indicated by different staining patterns of muscles (Boonmars *et al*., 2004).

Figure 1: Life cycle of a *Trichinella species*.



Adapted and redrawn from National centre of disease control USA (NCDC)

Life cycle duration

In female National institute of health (NIH) strain mice, expulsion of a primary infection of the nematode *T. spiralis* began on day 8 and was virtually completed by day 14 of infection. In secondary and tertiary infections, the number of larvae which established in the intestine was normal, but expulsion began on day 6 and was completed on day 10. In secondary and tertiary infections fecundity is depressed. The depression of fecundity occurred slightly in advance of worm loss (Kennedy, 1980). At day 6 p. i, adult *Trichinella* parasites were detected in the small intestine of reptiles (*Caiman crocodiles*, *Varanus exanthematicus*, *Python molurus bivittatus and Pelomedusa subrufa*) infected

with *T .papuae and T. zimbabwensis*, of chickens infected with *T. pseudospiralis* and mice infected with *T. spiralis, T. pseudospiralis, T. papuae, T. nativa, T. britovi, T. murelli, T. nelsoni* and *T. zimbabwensis (*Pozio *et al*., 2004*). Trichinella papuae and T. zimbabwensis* adult worms were identified and collected from the intestines of varans and caimans at day 60 p. i (Pozio *et al*., 2004).

In the characterization of *T. nativa* and *T. pseudospiralis* infections in the deer mouse (Peromyscus maniculatus), 77% and 23% of the 46% recovered *T. nativa* were found in the small and large intestines, respectively, on day 4 p. i. Thirty-one percent of the worms recovered on day 4 remained in the large intestine beyond day 20 p. i. Worms were embedded in the mucosa of the small intestine, caecum and colon (Poirier *et al*., 1993). Females recovered from the small and large intestines were indistinguishable in *in vitro* larval releases. Distension of the caecum and passage of loose stools were associated with the presence of worms in the large intestine. The ability of *T. nativa* to establish and thrive in the large intestine of deer mice was confirmed following intracaecal implantation of first-stage larvae (Poirier *et al*., 1993). Ninety-one percent and 9 % of the 35% *T. pseudospiralis* were found in the small and large intestines respectively on day 4 p. i. Although *T. pseudospiralis* established in the large intestine of deer mice, few worms remained beyond day 20. Females recovered from the small and large intestines were indistinguishable in *in vitro* larval releases. The large intestine may be a more important habitat for adult trichinae than previously recognized (Poirier *et al*., 1993).

Trichinella fecundity

When rats are experimentally infected with *T. spiralis*, on average only 50% of the larvae delivered to an animal can be recovered from the intestinal tract (Gagliardo *et al*., 2002). In cultures using cells in agarose, the majority of the surviving worms (25-50%) were unfertilized females and the factor limiting fecundity appeared to be male worm survival in the monolayer. Each time the investigators did not observe male worms in monolayers at the end of the experiment, they did not also observe fecund females. The males that were present appeared to be fully developed. However, in experiments in which all of the worms recovered from culture were evaluated, more males were found in the agarose than in the monolayer suggesting that perhaps male worms were less able to maintain their position in the epithelium hence improving survival of male worms is critical to enhancing fecundity in such a model system (Gagliardo *et al*., 2002).

Factors involved in intestinal physiology

Hormones

In a *T. spiralis* model of intestinal inflammation, cholecystokinin 8 (CCK-8) changed the inhibition in motor activity response of the jejunum seen in control animals to excitation (Torrents and Vergara, 2000). Torrents *et al*. (2002) demonstrated an exacerbated spontaneous motility and a significant increase of the excitatory response to CCK, both associated with a reversible inflammatory process and the hypertrophy of the muscle layers of *T. spiralis*-infected rats.

Nerve growth factor (NGF) is over-expressed in the small intestine as a consequence of *T. spiralis* infection and this neurotrophin plays an important role in the development of motor alterations (Torrents *et al*., 2002). Higher amounts of NGF in

inflamed tissue has also been described in other inflammatory models such as rheumatoid arthritis (Aloe *et al*., 1992), pancreatitis (Toma *et al*., 2000), and experimental colitis (Reinshagen *et al*., 2000) and has been associated with the increased nervous sensitivity during inflammation (Woolf *et al*., 1994).

Concentrations of the main molting hormone (ecdysteroid) in molting animals of between 10^{-5} - 10^{-10} M promoted molting in *T. spiralis, Ascaris suum and Dirofilaria immitis* (Gagliardo *et al*.,2002). Exogenous ecdysone stimulated microfilarial release in *Brugia pahangi*, meiotic reinitiation in the oocytes of *D. immitis*, egg laying in *Nippostrongylus brasiliensis* and insignificant ecdysis or development of *T. spiralis* (Barker *et al*., 1991; Simo *et al*., 1992; Gagliardo *et al*., 2002).

Acute administration of a steroidal anti-inflammatory dexamethasone before or early after experimental infection of mice with *T. spiralis*, even at the lowest dose, may induce long-lasting consequences detrimental to the infected host. Pre-treatment of mice with 0.1mg/kg dexamethasone significantly delayed adult parasite elimination from the small intestine and the mice harboured significantly more larval worms in the musculature than the control hosts (Bozic *et al*., 2000). As the amount of cortisone given to mice was increased, enteritis decreased and fecundity of adult worms increased. Injection of mice with cortisone caused retention of a greater percentage of adult worms in the anterior regions of the host's small bowel than in uninfected mice. By day 11 p. i, when adult worms in cortisone-injected mice were more widely distributed along the host's small intestine, fecundity of adult worms isolated from mice receiving more worms (500, 1000, or 2000 worms) attained levels previously seen only in worms from mice infected with only 250 *T. spiralis*. The extended longevity of adult worms in

cortisone-injected mice was accompanied by a severe reduction in fecundity after day 11 p. i. Addition of cortisone to the culture medium at concentrations above 0.1 mg was accompanied by a reduction in fecundity of adult worms (Stewart *et al*., 1982). The number of larvae in the infected mice decreased after the application of the female sex hormone oestrogen to males and male sex hormone testosterone to females (Figallova and Prokopic, 1988).

Lactobacillus

Viable *Lactobacillus casei* was found to induce host resistance which reduced worm establishment in *T. spiralis* infected rats (Bautista-Garfias *et al*., 1999). *T. spiralis* adult worms were expelled rapidly from the intestine of *L. casei* treated mice. An oral rehydration solution of reduced osmolarity (224 mosmol/l) combined with early administration of *Lactobacillus* GG is an effective treatment for acute diarrhea in young children and further reduction of osmolarity may not be beneficial (Rautanen *et al*., 1998).

Metabolites

Mettric and Podesa (1974) suggested that factors such as nutrition, hormonal or biochemical gradients along the length of the intestines are important for the establishment of a suitable microenvironment of *T. spiralis*. Alteration of the concentration of metabolites (glycogen, glucose, phosphocreatine/creatine, lactate betaine, succinate, alanine and taurine) by the tapeworm *H. diminuta* in the tissueś small intestines of rat have been reported *(*Novak *et al*., 1993).

Physico-chemical conditions

Differences in the distribution pattern of *Trichinella* worms are also seen when different sizes of inocula are used maybe as a result of differential flow rates in the small intestine. The flow rate is faster in the anterior small intestine than in the posterior (Sukhdeo and Croll, 1981). In heavy infections, the majority of larvae may not establish rapidly enough and are swept to the posterior where the flow is slower and penetration can be more leisurely. Whereas similar numbers establish in the anterior segments, regardless of inocula, in the heavy infections higher numbers establish more posteriad. There is a possibility that the parasites establish equally in the anterior small intestine but in animals with heavy infections the anterior becomes rapidly inhabitable and the rest migrate posteriorly as demonstrated in old infections by Kennedy *et al*.(1979). In Leghorn roosters, Brummermann and Braun (1995) reported a hydration state-related control of the retrograde colonic motility of which central osmoreceptors has no such effect on direct stimulation.

During a stable phase of infection, *T. spiralis* occur in the anterior half of the small intestine in female NIH mouse strain (Kennedy, 1980). During expulsion, living worms are found increasingly in more posterior parts of the gut but their fecundity does not vary with position (Kennedy, 1980). After direct inoculation into the posterior ileum, adult and larval *T. spiralis* remain in the posterior half of the small intestine. In this position, larvae established in normal numbers, grew and reproduced normally indicating that any part of the small intestine was a suitable site for *T. spiralis* and that expulsion is not merely due to a change in the position of the worms (Kennedy, 1980). Although higher establishments of *T. nativa* (56% vs. 46%) and *T. pseudospiralis* (52% vs. 35%)

were observed in CD-1 mice than in deer mice on day 4 p. i, neither was able to colonize the large intestine of the former (Poirier *et al*., 1993).

Physico-chemical conditions of the anterior small intestines were also reported to be optimal for *Trichinella*'s reproductive fitness and this is thought to exert a strong selective pressure on habitat selection behavior of the parasite. Adult female worms recovered from the jejunum of rats infected with 1000 larvae of *T. spiralis* were significantly more fecund than females recovered from the terminal ileum in the same infections. Worm fecundity was believed to be location-specific because adult females that were surgically implanted into the jejunum were significantly more fecund than those that were implanted into only the ileum (Sukhdeo, 1991).

Osmolality in uninfected host

The composition of upper jejunal contents after a meal reflecting that of the gastric contents was described by Ladas *et al*. (1983). Despite mixing with upper gastrointestinal secretions and transepithelial movement of fluid and electrolytes, osmotic and electrolytic equilibration of intestinal contents with plasma was not produced up to 2 h following a meal (Ladas *et al*., 1983). The decrease in osmolality of a hypotonic beverage from the stomach to the jejunum is attributable to greater solute flux than net water flux and the rise in osmolality of a hypotonic beverage from the stomach to the duodenum is attributable to maltodextrin digestion, sodium secretion and greater water than solute absorption The change in osmolality from the duodenum to the jejunum is attributable to increased electrolyte concentration, carbohydrate digestion and net water flux (Gisolfi *et al*., 1998).

In a model of transcellular flux, sodium actively absorbed in the apical portion of the cell provokes an increase in cell tonus and results in an osmotic gradient with the intercellular space (Schultz *et al*., 1974). The resulting influx of water tends to dilute the internal environment. In another model, the permeability of tight junctions is altered in the presence of sodium and an osmotic gradient, thereby leading to a flow of water in the mucosa-serosa direction, principally through the intercellular space (Schultz *et al*., 1974). Since water absorption and sodium transport in the jejunal mucosa are interdependent, these findings may be understood to represent a flux of liquids between the mucosa and serosa (Schultz *et al*., 1974; Armstrong, 1987).

The diarrhea in subjects deficient in lactase may result from an osmotic effect of the lactose itself or its poorly absorbed acidic products of fermentation (Weijers *et al*., 1961; Christopher and Bayless, 1971), possibly together with an alteration of sodium and water absorption due to the lowered colonic pH (Rousseau and Sladen, 1971). Apart from the increased osmotic effect, the pH in the proximal colon falls markedly (Brown *et al*., 1974) and larger doses may reduce stool pH. Weijers *et al*. (1961) inferred that the acidic products formed from lactose in the colon stimulate propulsion and suggested that lactulose might relieve constipation partly by stimulation of propulsion due to the lowered pH (Bennett and Eley, 1976).

Intestinal muscle contractility as a secondary factor

Intestinal inflammation as a result of *Trichinella* infection has been associated with functional motor changes and morphological alterations (Torrents and Vergara, 2000). Receptors in the stomach and the duodenum responds to volume, osmotic pressure, acids, fats, fatty acids, amino acids and control entero-gastric reflexes (Ehrlein

and Stockmann, 1998). *Trichinella* infection induces changes in the deeper neuromuscular layers of the intestine, accompanied by accelerated intestinal transit. Since the increased propulsion is observed in extrinsically denervated gut segments, the changes in propulsive activity reflect changes in those components of the motility apparatus that are intrinsic to the bowel wall, thus implicating enteric nerves and muscle (Bruce *et al.,* 1998; Alizadeh *et al*., 1987). Bruce *et al*. (1998) identified two distinct components of increased contractility in the mouse intestine after *T. spiralis* infection. There was an early phase that occurs rapidly, within 6 days of infection, followed by a smaller sustained phase that persists for up to 21 days. In athymic and SCID mice, the early phase was delayed several days and the sustained phase was reduced in magnitude. This attenuated profile of muscle contractility was accompanied by a delay in worm expulsion. Reconstitution of T-cell function in athymic mice was accompanied by restoration of the profile of muscle contraction seen in euthymic mice and by faster worm expulsion.

Blennerhassett *et al*. (1996) reported that the ability to expel *T. spiralis* from the small intestines is not related to the degree of granulocyte-dependent mucosal inflammation but is reflected in the magnitude of the accompanying increase in force generation by intestinal smooth muscle. The timing and magnitude of muscle changes are important in worm expulsion and there are both T-cell dependent and independent mechanisms that underlie the enhanced contractility of intestinal smooth muscle in the *Trichinella* mouse model (Bruce *et al.,* 1998). When the rate of propulsion of the gut is decreased such as with lomotil (diphenoxylate/atropine) treatment, *T. spiralis* larvae

establish more anteriorly than the controls and when propulsion is increased, they establish more posteriorly (Sukhdeo and Croll, 1981).

Proteinases and pH

Proteinases have been reported to be probably active in the processes of parasite transformation and counter-immunity. They have also been implicated in host invasion and parasitic nutrition as well as evasion of the host immune responses to the parasite (Auriault *et al*., 1981; McKerrow and Doenhoff, 1988; Robertson *et al*., 1989; Todorova, 2000).

The excretory/secretory products of parasitic helminths are known to contain a variety of enzymes including acetycholinesterases, superoxide dismutases and proteinases (Knox, 1994). *Trichinella* secretes a variety of proteinases during every phase of its life cycle (Parkhouse and Almond, 1985). Secreted exoenzymes, apyrase, 5'-nucleotidase, and adenosine deaminase active in the alkaline pH region, exhibiting a broad optimum between the values of 7.0 and 8.5, are used by *T. spiralis* to modulate both the availability and concentration of extracellular nucleotides (Gounaris, 2002). *In vitro* studies have also shown that the infective larvae of *T. spiralis* secrete proteinases predominantly of the serine type with azocollytic and elastolytic activities (Todorova, 2000). Degradation of azocasein by proteolytic enzymes in the larval excretory/secretory (ES) products occurs at a broad pH range with peak activity at pH 7 and high activities at pH 5 and 6. The collagenolytic activity was maximal at pH 5 while elastolytic was at pH 7 (Todorova, 2000).

Proteinases and cations

Magnesium ions are activators of *T. spiralis* secreted exoenzymes although the activity of 5'-nucleotidase is not absolutely dependent on the presence of the cation (Gounaris, 2002). In another *T. spiralis* study expression in *Pichia pastoris* resulted in the secretion of an active enzyme with the catalytic properties of both a Mg^{2+} dependent diphosphohydrolase/apyrase and a 5'-nucleotidase (Gounaris *et al*., 2004). The diphosphatase activity was dependent on the presence of Mg^{2+} and a reducing agent while the 5'-nucleotidase activity was enhanced by these additions (Gounaris *et al*., 2004).

Calcium ions cannot substitute for magnesium and indeed inhibit apyrase activity. Zinc is also inhibitory for both enzymatic activities (Gounaris, 2002). In the absence of divalent cations, significant 5'-nucleotidase activity but minimal apyrase activity are observed.

With proteinases likely to be active in the processes of parasite transformation and counter-immunity, these enzymes have been implicated in host invasion and parasitic nutrition as well as evasion of the host immune responses to the parasite (Auriault *et al*., 1981; McKerrow and Doenhoff, 1988; Robertson *et al*., 1989; Todorova, 2000).

Host cell nucleotides

Adenosine and inosine the products of the 5′-nucleotidase and adenosine deaminase are regulators of inflammation and have been shown to have immunomodulatory effects (Cronstein, 1994; Linden, 2001). Adenosine besides inhibiting platelet aggregation, suppresses the production of inflammatory cytokines *such* as IL-1, IL-12, tumour necrosis factor alpha, macrophage protein 1α by monocytes and macrophages and enhances IL-10 production (Hasko *et al*., 2000a; Kawashima *et al*.,

2000; Link *et al*., 2000; Nagasawa *et al*., 2001). Inosine has been shown to suppress inflammatory cytokine synthesis (Hasko, *et al*., 2000a; Marton *et al*., 2001). The role of extra cellular adenosine triphosphate (ATP) is more complex having both pro-inflammatory and anti-inflammatory effects such that although it enhances release of IL-10 from macrophages, it triggers mast cell chemotaxis and the oxidative burst in neutrophils. It is also known to suppress IL-12 and TNF-α release from macrophages (Kuroki and Minakami, 1989; McCloskey *et al*., 1999; Hasko *et al*., 2000b, c). Transfer and over expression of the IL-12 gene during Th_2-based nematode infection shifts the immune response toward Th_1 and delays worm expulsion (Khan *et al*., 2001).

Expulsion of the gastrointestinal nematode *T. spiralis* is associated with pronounced mastocytosis mediated by a Th_2-type response involving IL-3, IL-4, IL-5, IL-10 and IL-13 and negatively regulated by IL-18 (Urban *et al*., 1998; Helmby and Grencis, 2002). Interferon gamma (IFN-γ) a Th_1-cytokine involved in macrophage activation widely regarded as the major macrophage-activating cytokine is thought to be important in mice infected with *T. spiralis* (Pond *et al*., 1989). Phagocytic cells and natural killer cells are among the effector cell types providing innate resistance. During the early inflammatory response to an infection, regulatory interactions between these cell types are mediated by cytokines such as IFN-γ that regulate activation and migration of phagocytic cells and natural killer cells (Trinchieri, 1995).

Immunity

Studies have demonstrated that changes in intestinal physiology are influenced by the immune response of the host to the invading parasite (D'Inca *et al*., 1989; Harari and Castro, 1991; Vermillion *et al*., 1991). Surviving worms recovered from the small

intestines of IL-5 transgenic mice exhibit reduced fecundity, fail to grow and were less likely to occupy the preferred site of attachment in the anterior section of the small intestine, suggesting that parasite growth and development may be adversely affected in the small intestines of IL-5 transgenic mice (Butterworth, 1984)

Although the degree of pathology elicited depends upon the isolate and host concerned, the pathology associated with *Trichinella* infection is essentially biphasic. The initial inflammatory reaction is a response to the intestinal stage and the second reaction is an allergic and inflammatory response to the migratory larvae and muscle invasion (Stewart *et al*., 1987; Wakelin and Goyal, 1996).

It is suggested that priming for rapid expulsion of *Trichinella* requires two separate stimuli to the host (Bautista-Garfias *et al*., 1999). One is immunological and specific to the *Trichinella species* while the other is a locally acting priming of the intestine, which can be induced non specifically (Bell and McGregor, 1980). Larsh and Race (1975) suggested that worm rejection was as a result of an inhospitable intestinal environment created by the immune response.

Even though antibody responses are important in worm expulsion in some cases, the availability of specific monoclonal antibodies and gene targeted mice have suggested that antibodies and mast cells are not involved in a number of intestinal nematode parasites expulsion during a primary infection (Grencis, 1997). The expression of both NGF and its receptors by immune cells such as mast cells, lymphocytes and basophils was described previously (Leon *et al*., 1994; Otten *et al*., 1994; Levi-Montalcini *et al*., 1996; Sawada *et al*., 2000). In addition, a correlation between proliferation of vagal

pathways and hyperplasia of mast cells has been demonstrated in experimental parasite infection (Stead, 1992).

Numerous cellular changes occur in the intestines of mice and rats infected with *T. spiralis*. Cellular changes together with the development of immunity are dependent upon T-lymphocytes. Type 1 cytokine responses are detected in the earliest stages of *T. spiralis* infection in rats (Ramaswamy *et al*., 1996) and mice (Grencis *et al*., 1987; Ishikawa *et al*., 1998), however a type 2 cytokine response soon predominates and is essential for termination of the intestinal infection in mice (Urban *et al*., 2000).

Rats adoptively immunized with CD4+ T lymphocytes obtained from the thoracic duct of *T. spiralis* infected donors expelled adult worms in an accelerated manner (Bell *et al.,* 1987). Fractionation of transferred cells revealed that CD4+,OX22- (CD45RC-) cells confer protection together with eosinophilic influx into intestinal tissue, while CD4+,OX22+ cells are not protective and induce increased mast cell numbers (Wang *et al.,* 1990). CD4+,OX22- cells produce exclusive type 2 cytokines while CD4+,OX22+ cells produce IFN-γ in addition to type 2 cytokines (Ramaswamy *et al*.,1994). By infecting various immunodeficient mouse strains, as well as gene transfer to the intestine, T-lymphocytes, and in particular the CD4+ve subset were found to be responsible for altering smooth muscle function. However, eosinophils as well as the cytokine IL-4 may also be involved (Vallance and Collins, 1998). Despite the abatement of cellular changes including mast cell proliferation, nitric oxide and villus blunting, adult worm rejection was normal (Appleton *et al*., 2001), even though evidence supports a role for mast cells in worm rejection in mice and not in rats (Bell *et al*., 1987).

The hypothesis of involving alteration of gut physiology still remain the most likely model. The leak lesion hypothesis of host protection suggested that the initial phase of the anti-parasite response consist of antigen specific T-cells inducing Ig E production by B-cells which then sensitize mast cells by binding the surface of the cell through Fc∈R1 receptors. Worm expulsion was correlated to the time of appearance of specific intraintestinal IgE directed against adult worms. LEWIS rats had detectable antibodies at day 12 p. i while infected PVG rats first had detectable levels at day 14 p. i (Negrao-Correa *et al*., 1999). During a *T. spiralis* infection, total IgE levels in the intestinal lumen were consistently higher in LEWIS and LOU rats (rats strains that eliminate *T. spiralis* worms earlier in the infection) than in PVG, AO and WKA/H strain rats (Negrao-Correa *et al*., 1999). Interestingly, it is unclear how mast cells are activated during nematode infection, as granulation can occur in the absence of the classical IgE/Fc∈R1Fo interaction although this has also been reported to be modulated by P1 receptor activation (Tilley *et al*., 2000; Linden, 2001). Following subsequent exposure to antigen, IgE mediated degranulation of mast cells occurs inducing type1 hypersensitivity in the gut through the release of the pharmacological mediators such as histamine, cell specific serine proteases, cytokines, leukotrienes and prostaglandins. This non-specific inflammatory phase is thought to make the intestinal microenvironment unsuitable for nematode feeding and survival or directly to damage the parasite hence inducing expulsion (Wakelin, 1978 and 1993). Deletion of the mouse mast cell protease-1 gene is associated with significantly delayed expulsion of *T. spiralis* (Knight *et al*., 2000).

Physiology of poultry gastrointestinal tract

Although the anatomy and physiology of the poultry gastrointestinal tract (GIT) is sufficiently different when compared to monogastric mammals, the intestine and more generally the GIT is a very complex organ as shown in figure 2. It is the obliged passage of the nutrients that support basic metabolism, growth and maintenance, supplying the resources to support the immune, skeletal and nervous systems (Ferket, 2000).

Intestines

The absorptive surface of the small intestine is covered by a layer of mucus secreted by goblet cells. The secreted mucins and thickness of the adherent layer influence nutrient digestion and absorption processes as well as the functionality of the mucosa (Sklan *et al.,* 2001). Lipase activity is markedly inhibited by a decrease in the ratio of conjugated/unconjugated bile salts and the magnitude of inhibition is pH-dependent, showing a more pronounced inhibition at low pH. This may also play an important role in the infectivity of adult *T. zimbabwensis*.

In mammals, the kidney and the large intestine are the major regulators of body electrolyte and water homeostasis. As birds do not have a urinary bladder, the urine is emptied into the cloaca, and retrograde flow carries urine into the coprodeum, the colon (rectum), and cecum. As a result, the epithelia of the lower intestine process the ileal and ureteral outflow (Carmen De La Horra *et al.,* 2001).

Crop and stomach

Although substantial amylolysis occurs in the crop and absorption of sugars appears possible but minimal, the crop is not essential for normal growth when access to food is sufficient (Pinchasov and Noy, 1994). The oxynticopeptic cells found in birds

secrete both HCL and pepsinogen. According to Joyner and Kokas (1971), the pH of gastric contents is 2.6 although it is normally above 2.6 due to the presence of ingesta. Higher pH values have been reported when measurements are made on live birds. Age has no effect on pH of the digestive tract (Herpol, 1966). Acid secretion of chickens is high relative to mammals, possibly because of the rapid digestive transit time (Denbow, 2000). The basal acid secretary rates of chickens and mammals and the gastrointestinal pH values of avian species are shown in table 1 and 2 respectively.

Table1: Basal gastric secretory rates of the chicken in comparison with those of mammals

	Chicken	**Rat**	**Monkey**	**Man)**
Volume ml/h	15.4	1.3	5	60
	8.8	3.7	2	0.86
Acid concentration ml/kg BW/h	90	66	60	36
Acid output mEq/ml	1.36	0.09	0.3	2.16
Pepsin Concentration units/h	247	600	365	1035
Pepsin output units/h	4256	780	1825	62100
PU/kg BW/h	2430	2230	730	862

Values from Denbow, 2000 in Whittow G C, editor: Sturkies Avian Physiology,California, Academic Press, based on results from [a]Sturkie 1976 based on work of Farner 1942; [b] Herpol 1966 ;[c] Herpol and van Grembergen 1967; [s] Simon and Versteeg1989 in Vanbelle 1999.

Table 2: pH of contents of the digestive tract of avian species and mean duration of transit time of ingesta in chickens.

	Transit Time duration (min) in chickens	**Chicken**	**Pigeon**	**Pheasant**	**Duck**	**Turkey**
Crop	50	4.51 5.5[s]	6.3 [a] 4.28	5.8	4.9	6.0
Proventriculus	90	4.8	1.4 [b]	4.7	3.4	4.7
Gizzard		4.74 [c] 2.50	2.0	2.0	2.3	2.2
Duodenum	5-8	5.7-6.0;5-6[s]; 6.4 [c]	6.4 [c] 5.2-5.4	5.6-6.0	6.0-6.2	5.8-6.5
Jejunum	20-30	5.8-5.9; 6.5-7.0[s]; 6.4 [c]	6.4 [c] 5.2-5.4	5.6-6.0	6.0-6.2	5.8-6.5
Ileum	50-70	6.3-6.4 7-7.5[s]	6.8 [b]	6.8	6.9	6.8

1.Values from Denbow, 2000 in Whittow G C, editor: Sturkies Avian Physiology,California, Academic Press, based on results from [a]Sturkie 1976 based on work of Farner 1942; [b] Herpol 1966 ;[c] Herpol and van Grembergen 1967; [s] Simon and Versteeg1989 in Vanbelle 1999.

2. pH values with an [s] and mean duration transit time were taken from allmesh fed chickens *ad libitum* during 6 weeks.

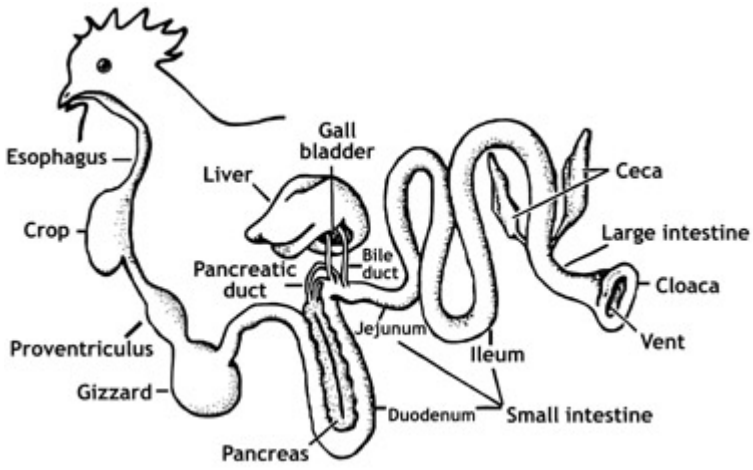
Intestinal cations as partial determinants of osmolality

The absorption and secretion of sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the gastrointestinal tract of broilers was characterised by van der Klis *et al*. (1990). Phosphorus (P^{+}), Ca^{2+} and Mg^{2+} are secreted in the duodenum in addition to the Mg^{2+} which is secreted in the ileum and rectum (Denbow, 2000). Dry matter spends approximately 25% of its time in the jejunum which is the major site of absorption of these minerals with some absorption of Ca^{2+} and Mg^{2+} occurring in the gizzard. Little absorption occurs beyond the jejunum (Denbow, 2000).

Effect of feed on pH

Whole wheat-fed chickens have increased relative weight of the pancreas and gizzard in addition to the reduced pH in the gizzard contents, increased ileal viscosity and a greater pepsin activity, which may increase the denaturation and hydrolysis of dietary proteins (Gabriel *et al*., 2003; Engberg *et al*., 2004). Addition of xylanase to whole wheat results in lower activities of pancreatic amylase, increased chymotrypsin and lipase activities as compared to pellet-fed broilers (Engberg *et al*., 2004). With a fine particle diet, the feed is less exposed to low pH and proteases in the gizzard and ingested feed appears more quickly in the duodenum as a suspension of relatively unchanged particles (Hill, 1971). The poorly digested feed particles in the upper small intestine may play a role in the development of aberrant bacterial populations such as suggested by Cumming (1994). Gabriel *et al*. (2003) reported that diet had no difference in the pH of the contents of the jejunum or ileum.

Figure 2: Diagrammatic representation of the digestive tract of a broiler chicken



from Parkhurst and Mountney (1987).

MATERIALS AND METHODS

Parasite strain

The *Trichinella zimbabwensis* strain used in the experiment was maintained under laboratory conditions by periodic passage in rats (*Rattus norvegicus*). The number of *Trichinella zimbabwensis* larvae in muscle was determined by visual enumeration using a trichinoscopy. The adult *Trichinella zimbabwensis* parasite used for length and sex determination was from experimentally infected rats.

Experimental animals

Ten week old male rats (*Rattus norvegicus*) bred and maintained at the animal house unit of the University of Zimbabwe and three weeks old chicks bought as one day old chicks (*Gallus gallus)* from Ross breeders (Pvt.) Ltd. Zimbabwe and raised in the animal house unit of the University of Zimbabwe were used in the experiment. All animals selected were healthy.

Animal feed

The chicks were fed with chick starter mesh bought from Agri-foods a parent company of Ross breeders while the rats were fed with mouse comproids from SAFCO a subsidiary company of National foods (Pvt.) Ltd Zimbabwe.

Infection of animals

The chicks (*Gallus gallus)* were divided into groups by simple random sampling as shown in Table 3 and infected at an age of three weeks. Each chicken was fed 0.6g of fresh meat containing approximately 1700 *Trichinella* first stage larvae (L_1).

Experimental rats selected by simple random sampling and allowed to adapt to the experimental environment for one week were each fed 1g per os of fresh rat meat containing approximately 1000 *Trichinella* first stage larvae (L_1).

Table3: Number of rats(R) and chickens (P) slaughtered at different days post-infection

Groups	**Number(s)**	**Day 2**	**Day 5**	**Day 7**	**Day 10**
1.Rats (uninfected)	20	4	4	4	4
2. Rats (infected group)	25	5	5	5	5
3. Chickens (uninfected)	12	6	6	-	-
4. Chickens (infected group)	12	6	6	-	-

The experimental animals were fasted overnight before group 2 and group 4 animals were fed muscle tissue containing 1000 infective L_1 stage larvae following methods described by Kapel *et al*. (1998). Groups 1 and 3 were uninfected controls. Although water was available *ad lib*, to minimize possible effects of food and diurnal variation on pH and osmolality, food was withdrawn for 17 hours starting at 14H30 from the experimental animals and reintroduced *ad lib* at a standardized time (07H30) before sacrificing the rats and chickens 3 hours later. The rats were first anaesthetized with ether while the chickens were stunned before sacrificed through decapitation. The different groups of rats were sacrificed on day 2, 5, 7 and 10 and chickens at days 2 and 5 p. i respectively.

pH and osmolality measurements

The study was based on the assumption that the anterior half of the distance from the pyloric sphincter to the ceca represented the anterior portion of the small intestine while the remaining posterior half represented the posterior portion. Using a calibrated ruler, the anterior half of the small intestines was separated from the posterior half. The contents of each of these segments were seperately collected into 1.5 ml eppendorf tubes for the measurement of pH and osmolality according to the method described by Skadhauge *et al*. (2002). Each portion of the small intestine was divided into three sub-portions. Representative samples of 0.5 ml of the intestinal contents were collected from each of the sub-portion into eppendorf tubes which were tightly closed with their caps and placed in crushed ice to minimize evaporation. These labeled samples of 1.5 ml from rats sacrificed on day 2, 5, 7 and 10 and chickens sacrificed at day 2 and 5 were each time centrifuged immediately (less than 10 minutes) in eppendorf tubes at 13000 rpm

(3325 x g) at 0 °C for 35 minutes in a refrigerated centrifuge (Biofuge fresco, Germany). The harvested supernatant was kept at 0 ^{o}C for immediate measurement of pH and osmolality. The pH of the intestinal contents was measured using a Crison pH meter GLP21 (Carburos metalicos, Barcelona, Spain). Osmolality was determined on a 50-µl sample of supernatant in an eppendorf tube by freezing point depression (Osmomat 030, Gonotec, Germany).

Recovery of adult *Trichinella zimbabwensis* for length and sex determination

The intestinal segments were processed for recuperation of adult worms according to the method described by McCracken (1982). Each segment was slit open longitudinally and placed in a separate dish. The segments were incubated in 0.85% saline at 37 °C for 4 h before the adult worms which had migrated from the intestinal wall into the saline were recovered. The parasites were counted and their sex status noted with the aid of a stereo microscope. Parasites with a twin terminal appendages and papillae were considered as males while those with a single uterus filled with developing eggs in its posterior region were regarded as females. After the samples were fixed in 70% alcohol their length was measured with the aid of a calibrated stereo microscope.

Statistical analysis

The effect of infection was determined by analysis of variance (ANOVA) using the general linear model of the Statistical Analysis Systems. Differences were considered significant at a *P* value of <0.05 and all values were expressed as mean ± S.E

RESULTS

Health of experimental animals

No visual clinical abnormalities were observed in the infected and control rats and chickens.

Recovery of adult worms

The number of adult *Trichinella* parasites recovered from rats in the infected groups ranged from 91-416 at day 2, 60-282 at day 5,116-282 at day 7 and 145-255 at day 10. There was a variation in the establishment of *T. zimbabwensis* from each individual rat within a group. The largest number of recovered parasites from an individual rat was recorded at day 2 (416 parasites) (Figure 3). The distribution of male and female parasites in the anterior and posterior section of the small intestine in rats is shown in Figure 4. The number of male parasites increased from day 2 to day 10 in both the anterior and posterior sections.

In chickens, adult *Trichinella* parasites were observed only in the anterior segment of the small intestine. At day 2 p. i, one male and nine female parasites were observed from the infected group (n=6) and only 5 females were observed at day 5 p. i. No eggs were microscopically observed from the recovered female parasites in the chicken group.

Figure 3: The number of adult *Trichinella zimbabwensis* recovered from the small intestine of individual rats at days 2, 5, 7 and 10 after oral infection with 1000 larvae/rat

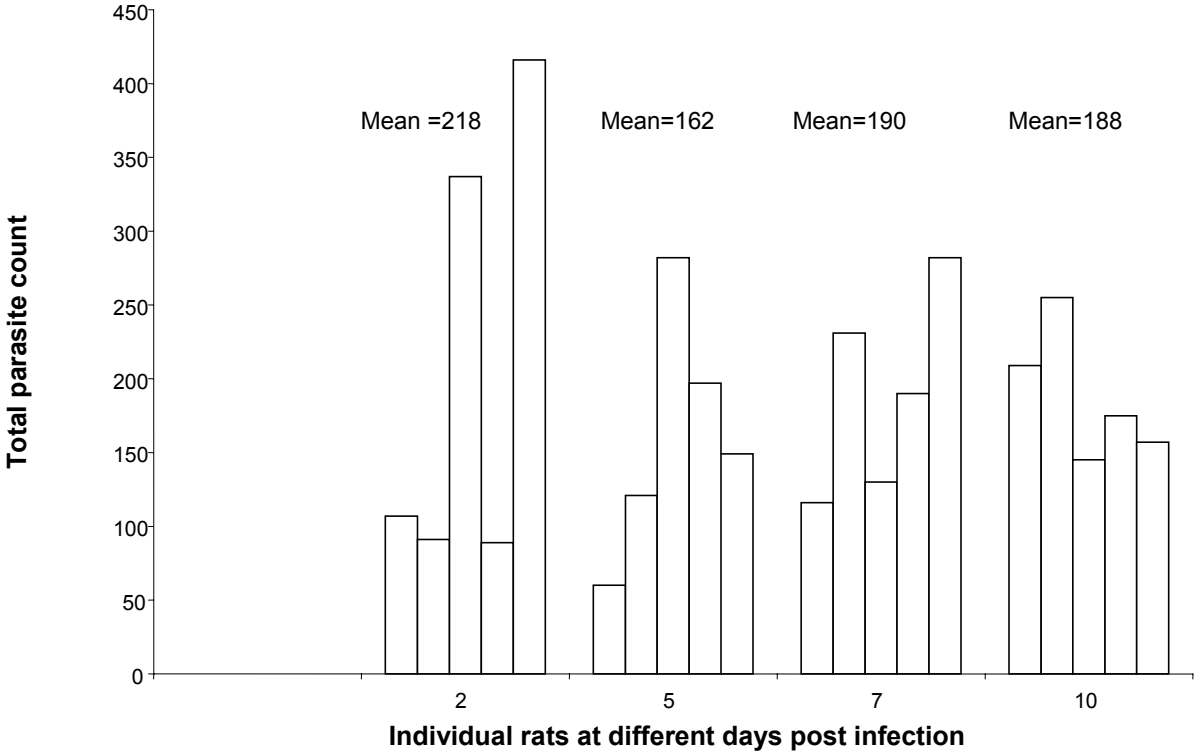
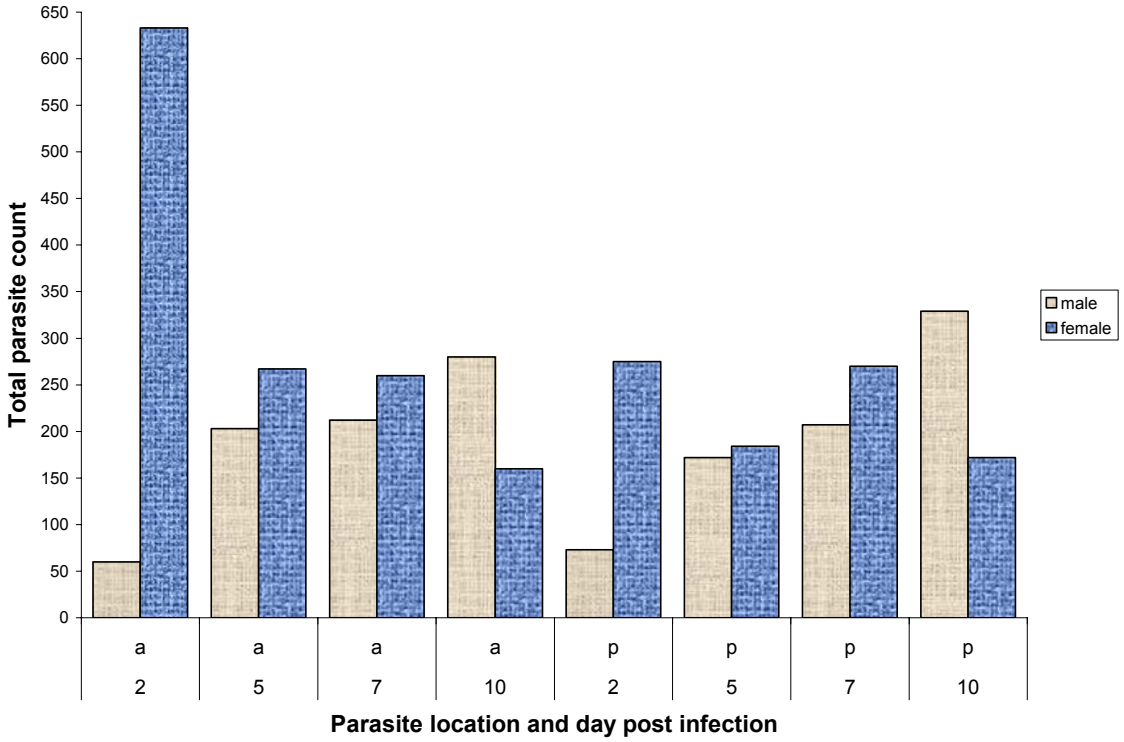


Figure 4: The population dynamics of *Trichinella zimbabwensis* in the anterior (a) and posterior (p) section of the small intestine of rats at day 2, 5, 7 and 10 after oral infection with 1000 larvae/rat



Adult parasite length

Mean length of randomly sampled *T. zimbabwensis* are shown in Table 5. Significant increases ($p<0.05$) in female parasite length was observed from day 2 to day 10 p. i. There was a significant increase ($p<0.05$) in the length of male parasites between day 5 and 7. A significant difference ($p<0.05$) in male and female length was observed with males being generally shorter than females. Overall mean parasite lengths in the posterior (1.6±0.03mm) and anterior (1.5±0.03mm) sections did not differ significantly.

Table 4: Mean length (±se) in millimeters (mm) of male and female *Trichinella zimbabwensis* recovered from the small intestine of rats at days 2, 5, 7 and 10 after oral infection with 1000 infective larvae/rat.

Days post infection	**Mean parasite length (N)**	
	male	female
2	0.9 ± 0.06^{a} (28)	1.5 ± 0.04^{c} (54)
5	0.8 ± 0.09^{a} (14)	2.0 ± 0.04^{d} (31)
7	1.2 ± 0.11^{b} (8)	2.3 ± 0.04^{e} (30)
10	1.2 ± 0.07^{b} (19)	2.5 ± 0.04^{f} (27)

N = sample size

Values with different superscript within a column are significantly different $P< 0.05$

pH

The pH levels of the small intestinal contents of infected and control rats are shown in Table 5. A significant increase in pH ($p<0.05$) in the anterior segment was observed at day 5 p. i. In the posterior segment of the infected, there was a significant decrease ($p<0.05$) in pH at day 2 and a significant increase ($p<0.05$) at day 5, 7 and 10 p. i when compared to the controls.

In chickens, the pH in the infected group was 6.3±0.07 and 6.2±0.07 at day 2 and day 5 p. i respectively, while that of the control group was 6.2±0.05. There were no significant pH differences ($p>0.05$) between the anterior, posterior, infected and control segments of the small intestine in chickens

Osmolality

Table 6 summarises osmolality levels measured from supernatant collected after centrifugation of the intestinal contents from *T. zimbabwensis* infected rats and non-infected rats. The osmolality value of the anterior segment of the small intestine of the infected rats increased significantly ($p<0.05$) at day 2 p. i before declining at day 5 p. i when compared to the control group (Table 6). In the posterior section, osmolality was significantly increased in the infected as compared to the controls at day 2 p. i. There were no significant changes in osmolality in the posterior section of the small intestines of infected rats at day 5, 7 and 10.

In chickens, osmolality levels of 452.0±29.09 at day 2 and 449.2±29.09 at day 5 in the anterior portion and 420.8±29.09 at day 2 and 400.2±26.55 at day 5 in the posterior portion were observed in the infected groups. The increase in average small intestinal

osmolality levels in chickens from 404.1±15.33 at day 2 to 424.7±19.69 at day 5 p. i, was not significant ($p>0.05$).

Table 5: Means of pH levels of the anterior and posterior segments of the small intestine in rats at 2, 5, 7 and 10 days after oral infection with 1000 larvae/rat of *Trichinella zimbabwensis*

Day p. i	**Number of rats**		**Intestinal contents pH**			
	control	**infected**	**Anterior**		**Posterior**	
			Control	**Infected**	**Control**	**Infected**
2	4	5	6.1 [a]	6.2 [a]	6.3 [ac]	5.7[d]
5	4	5	6.1 [a]	6.6[bc]	6.3 [ac]	6.9[b]
7	4	5	6.1 [a]	6.3[ac]	6.3 [ac]	6.6[bc]
10	4	5	6.1 [a]	6.3[ac]	6.3 [ac]	6.6[bc]
Standard error			0.08	0.15	0.01	0.15

Values with different superscript letters within a column are significantly different (P< 0.05)

Values within a row without a common superscript letters are significantly different ((P<0.05)

Table 6: Means of osmolality (mosmol kg^{-1}) levels of the anterior and posterior segments of the small intestine in rats at 2, 5, 7 and 10 days after oral infection with 1000 larvae/rat of *Trichinella zimbabwensis*

Day P.I	**Number of rats**		**Intestinal contents osmolality**			
			Anterior		**Posterior**	
	Infected	**control**	**Control**	**Infected**	**Control**	**Infected**
2	5	4	476.3 [a]	777.3±48.10[b]	422.8 [a]	602.0±48.10[c]
5	5	4	482.6 [a]	357.8±43.01[a]	424.6 [a]	371.6±43.01[a]
7	5	4	479.8 [a]	368.3±48.10[a]	419.4 [a]	437.3±48.10[a]
10	5	4	481.5 [a]	447.8±48.10[a]	422.6 [a]	458.4±43.02[a]
Standard error			19.50		26.68	-

Values with different superscript letters within a column are significantly ($P<0.05$) different

Values within a row without a common superscript letters are significantly ($P<0.05$) different

DISCUSSION

pH

Previous studies have indicated that immune rejection of worms in secondary infection involves physiologically and presumably immunologically distinct early and late responses, with each response having a different developmental stage of the parasite as its target (Stewart *et al*., 1986). In the present study, small intestine luminal pH was altered by the presence of *T. zimbabwensis*. In the anterior segment, marked alteration of pH was observed in infected rats at day 5 p. i. A significant decrease in pH at day 2 p. i followed by an increase at days 5, 7 and 10 p. i in the infected group when compared to the control was observed in the posterior segment. el-Temsahy (2001) reported that raising the gastric pH led to a significant increase in *T. spiralis* adult worm count with an increase in their fecundity both *in vivo* and *in vitro*. On the other hand, lowering gastric pH prior to infection led to a reduction of the adult worm count and to their inability to give birth to newborn larvae (el-Temsahy, 2001). Gastric acid induced by insulin in a dose dependant manner was reported to be responsible for the posteriad migration of *H. diminuta* (Cho, 1984).

Dobson (2004) discovered that pH alone had little effect on exsheathment of *Oesophagostomum columbianum*, except at a value of 6.5, when 10% of the *O. columbianum* larvae exsheathed after 8 h. Although variant cells and animal species do not have the same optimal pH, it is believed that optimal pH has an effect on the survival of cells *in vitro* by adjusting the intracellular enzymes and proliferation factors (Ran *et al*., 2002).

Trichinella spiralis secretes a variety of proteins during every phase of its life cycle and can modulate both the availability and concentration of extracellular

nucleotides from the host by means of secreted exoenzymes (Gounaris, 2002). Apyrase, 5'-nucleotidase and adenosine deaminase are active in the alkaline pH region, exhibiting a broad optimum between the values of 7.0 and 8.5 (Gounaris, 2002). *In vitro* studies have shown that the infective larvae of *T. spiralis* secrete proteinases predominantly of the serine type with azocollytic and elastolytic activities (Todorova, 2000). Degradation of azocasein by the larval excretoty/secretory proteolytic enzymes occurs at a broad pH range with peak activity at pH 7 and high activities at pH 5 and 6. The collagenolytic activity was maximal at pH 5 while elastolytic was at pH 7 (Todorova, 2000). The observed luminal pH range in this study of 6.2-6.6 for the anterior and 5.7-6.9 for the posterior in rats fits well with the peak and high activities of the secreted *Trichinella* proteinases which are also likely to be important for the survival of the *T. zimbabwensis* nematode. These enzymes have been implicated to be active in the processes of parasite transformation and counter-immunity, host invasion, parasitic nutrition as well as evasion of the host's immune responses to the parasite (Auriault *et al*., 1981; McKerrow and Doenhoff, 1988; Robertson *et al*., 1989; Todorova, 2000).

Although no marked establishment of *T. zimbabwensis* was observed in chickens, the pH range at days 2 and 5 p. i is within the survival range reported for most intestinal parasites. There is a likelihood of the pH accommodating establishment of the adult *Trichinella*, an indication that there are other probably physiological factors that are detrimental to the survival of the parasite in the small intestine of chickens.

Although there were no parasites recovered from the crop, proventriculus and gizzard at days 2 and 5 p. i, these organs are likely to contribute to the establishment of the parasite considering their pH levels. In non-infected chickens pH is reported to be

4.51 in the crop, 4.8 in the proventriculus and 2.5-4.74 in the gizzard (Denbow, 2000). A higher pH in the gizzard may limit its functionality (Cumming, 1994). When the larvae are ingested orally, the transit time from the crop to the duodenum under these unfavourable conditions is approximately 2 h 20 min (Vanbelle, 1999). It is possible that the mechanism of establishing and maintaining some degree of physiological adaptation that should occur between the parasite and the habitat is disturbed thereby limiting the survival of the parasite in the host even in the absence of evolutionary pressures (Holmes and Price, 1986).

Osmolality

In the present study, changes in osmolality of infected rats were observed at day 2 p. i in the anterior and posterior intestinal sections. *In vivo* osmolality flactuations in the small intestine due to changes in absorption rates in rats are prevented through effective control of diurnal fluctuations in quantity and osmolality of food reaching the small intestine from the stomach (Ferraris *et al*., 1990). Osmolality results obtained from this study, except at day 2, were lower than the reported range of 600 to 800 obtained from the duodeno-jejunal contents of rats fed with normal solid food while water was available (Osaka *et al*., 2001). Rises in duodenal osmolality (peaking at 430 mosmol kg^{-1}) associated with meals, and persisting for hours, have been reported to occur in pigs (Houpt, 1991). Osmolalities of the fluid from the different segments of the digestive tract of a rabbit was reported to be similar at 331 mosmol kg^{-1} although slightly hypertonic to the blood plasma of 297 mosmol kg^{-1} (Mongin *et al*., 1976).

In the present study osmolality in infected chickens ranged between 400 and 420 in the posterior section and 449 and 452 mosmol kg^{-1} in the anterior section. Previously,

Mongin *et al*. (1976) reported osmolality of: crop 537, gizzard 312, duodenum 571, proximal jejunum 650 distal jejunum 573, proximal ilium 514 and distal ilium 451 mOsm in non infected hens. Besides a decrease in net lumen-to-tissue fluid movement during primary and secondary infection with *T. spiralis*, changes in osmolality as a result of disruption of fluid secretion was associated with rapid expulsion of challenge *T. spiralis* in rats (Castro *et al*., 1979). Net lumen-to-tissue fluid movement was unaltered when rats were infected with (7×10^3 larvae/rat) and examined 30 min later. Five days after primary infection net secretion occurred at a rate of 45 µl/h per cm and the net absorption equivalent to the pre-infection level was observed 30 days p. i (Castro *et al.,* 1979). The response was initiated faster in previously infected hosts. The rapid induction of net fluid movement in the direction of secretion upon secondary contact with the parasite was associated temporarily with prevention of worm establishment (Castro *et al*., 1979). Elsewhere, artificial increases in fluid secretion through administration of serotonin reduced the establishment of *T. spiralis* infection in naive rats (Zhang and Castro, 1990). Hallback *et al*. (1980) found out that when the lumen of the human intestine was exposed to an isotonic glucose-electrolyte solution, an osmotic gradient along the length of the villus was revealed with an osmolality of ~700mosmolkg^{-1} at the tip and an osmolality that equated with that of the plasma at the base (Gisolfi *et al*., 1998).

The location of the parasite in the intestinal mucosa may be a critical determinant of whether or not an intestinal response occurs (Schultz *et al*., 1974; Negrao-Correa *et al*., 1999). Loeschke *et al*. (1970) demonstrated using an isolated loop of the small intestine of toads that an osmotically induced liquid flux is more intense in the mucosa to serosa direction than in the opposite one. Ultrastructural studies carried out by

the same authors showed that during the absorption of liquids, the lateral intercellular space is distended. When an osmotic flux occurs in the opposite direction, the lateral intercellular space collapses, thereby diminishing the influx of liquids. Similar results were obtained by Wright *et al*. (1972) who demonstrated distention of the intercellular space when a hypertonic solution was applied to the serosa. When the mucosa was in contact with a hypertonic solution, the intercellular spaces collapsed, the resistance of the tissue increased and the permeability to solutions diminished.

Receptors in the stomach and the duodenum respond to volume, osmotic pressure, acids, fats, fatty acids and amino acids, and control entero-gastric reflexes (Ehrlein and Stockmann, 1998). Hyperosmolar and HCL solutions induce more frequent and large amplitude, segmental contractions whereas lipid and bile induce fewer and smaller amplitude contractions. The volume, the pH, the osmolar and the nutrient make up of the infusate may each influence the duodenal motor responses (Rao *et al*., 1996). In a study of 43 NaCl infusions, motility was increased in 24, seven of them with a typical migratory complex, phase III. In 17 cases, non propagated contractions increasing in a cephalo-caudal direction were noted with the latter likely to be related to delay gastric emptying associated to hyperosmotic loads (Defilippi *et al*., 1991).

Inhibition of gastric emptying by hyperosmolar mannitol depended primarily on duodenal resistance, while the inhibitory effect of hyperosmolar glucose depended on nutrient-specific feedback on the stomach more than duodenal resistance (Lin *et al*., 1993). Sukhdeo and Croll (1981) observed differences in the distribution pattern of *T. spiralis* when different sizes of inocula were used probably as a result of differential flow rates in the small intestine. Changes in motility likely combine with other mechanisms,

including increased fluid secretion to evict parasites from the gut rendering the intestine an inhospitable environment for the parasite, leading to a decrease in fecundity and expulsion of the parasite (Wakelin, 1993; Urban *et al*., 1995; Collins, 1996).

*Trichinella l*ength and recovery of adult worms

Male length ranged from 0.9±0.06 at day 2 p. i to 1.2±0.07 at day 10 p. i and female length ranged from 1.5±0.04 at day 2 p. i to 2.5±0.04 at day 10 p. i respectively. Sohn *et al*. (2000) reported the larvae length of 0.775-1.050 (average 0.908) mm. Urquhart *et al*. (1998) reported *T. spiralis* adult males to be approximately 1.0 mm long while females measured approximately 3.0 mm in length. Our findings for the male length range are in agreement with the previously reported results for *T. spiralis*. The difference in length between female *T. spiralis* and female *T. zimbabwensis* could probably be accounted by the fact that Urquhart *et al*. (1998) did not indicate at what stage of the life cycle the parasite measurements were taken.

In this study, the mean adult *T. zimbabwensis* recovery percentages were 21.8% at day 2, 16.2% at day 5, 19.0% at day 7 and 18.8% at day 10. Although methodological limitations may reduce the *Trichinella* recovery, recovery percentages have been seen to vary widely within and between experiments with a range of 5-19% reported in rats (Nuñez *et al*., 2002). Although the intestinal worm recovery was lower compared to the infection dose in this experiment, it compares well with the figures reported for *T. spiralis* in previous studies. In other animals, recovery percentages for *T. spiralis* were reported to be in the range of 2-8% in guinea pigs (Roth, 1938),3-62% in mice (Belosevic and Dick, 1979; Sukhdeo and Meerovitch, 1980; Bell *et al*., 1985; de Vos *et*

al., 1992; Ferens and Kayes, 1994; Ross *et al*., 1994; Wakelin *et al*., 1994; Goyal *et al*., 2002) and 5-22% in pigs (Murell, 1985; Marti and Murell, 1986).

Trichinella establishment in chickens

Trichinella zimbabwensis can establish in chickens inoculated with a dose of 1000 larvae as shown by the recovery from the small intestine of 10 adult parasites at day 2 p. i and five at day 5 p. i. Low susceptibility to *T. pseudospiralis* in ostriches has already been reported (Piergilli Fioretti *et al*., 2001). The low susceptibility of birds to trichinellosis is probably due to physiological factors that resist the activation of infective larvae to the adult stage and the resulting poor reproductive capacity index. Although muscle tissue of the legs in ostriches is the preferential site of *T. pseudospiralis* larval distribution, *T. spiralis* is only found in the bird's muscle tissue after inoculation with a high number (80 000) of larvae (Piergilli Fioretti *et al*., 2001).

CONCLUSION AND RECOMMENDATION

Osmolality increased at day 2 p. i while pH increased at day 5 p. i in the anterior section and decreased at day 2 as compared to control in the posterior section of the small intestine of rats in the presence of adult *T. zimbabwensis*. The alterations are probably crucial for the establishment and survival of the parasite.

In this experiment, it is difficult to explain whether the pH and osmolality changes were as a result of the host responding to the presence of the parasite or the parasite inducing the changes so as to establish a suitable microenvironment. In the exploration of physiological factors and the infectivity of *T. zimbabwensis*, *in vitro* studies will give more valuable information since some factors can easily be controlled while investigating the mechanisms and effects of specific physiological factors on specific vital parameters of the parasite that determines its infectivity for example parasite reproductive organs.

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