Experimental Parasitology 126 (2010) 156–160

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Peptidases of pinworms *Syphacia muris* and *Passalurus ambiguus*

Jaroslav Vadlejch [a,*], Andriy Lytvynets [b], Ivana Jankovská [a], Iva Langrová [a]

[a] Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 957, 165 21 Prague 6 – Suchdol, Czech Republic
[b] Department of Laboratory Animals Breeding and Hygiene, Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i., Vídeňská 1083, 142 20 Prague 4 – Krč, Czech Republic

ARTICLE INFO

Article history:
Received 26 February 2009
Received in revised form 1 February 2010
Accepted 19 April 2010
Available online 28 April 2010

Keywords:
Peptidases
In vitro maintained
Pinworms
Nematodes
Laboratory animals

ABSTRACT

In this first report about pinworms peptidases we primarily characterize peptidases released during in vitro maintenance of two common pinworms of laboratory animals – *Syphacia muris* and *Passalurus ambiguus*. The peptidase activity obtained using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) showed the presence of peptidases from *S. muris* with a wide range of molecular size (25–110 kDa), which degrades gelatin and mucin at alkaline pH levels. *P. ambiguus* released serine and aspartyl peptidases degrading gelatin at all tested pH (3, 5, 7, and 9) and at acidic pH *Passalurus* released aspartyl and cysteine peptidases which are active against mucin.

1. Introduction

Members of the Oxyurida, the so-called pinworms, are parasitic nematodes which are localized in posterior parts of the gastrointestinal tract of vertebrates and herbivorous arthropods. *Syphacia muris* (Yamaguti, 1941) is a common pinworm of rats. Adult worms inhabit the cecum and colon. Eggs are deposited by the female on the perianal area of the host or in the colon. Infection of the rat occurs by ingestion of embryonated eggs from the perianal area or the cage environment (Stahl, 1963). *Passalurus ambiguus* (Rudolphi, 1819) infects domestic and wild rabbits and hares, and its distribution is worldwide. This pinworm, inhabiting the cecum and colon of its hosts, has a direct life cycle similar to *S. muris* (Owen, 1972; Rinaldi et al., 2007). The problem of pinworm infection in lab rodents and rabbits has been prevalent since the early days of lab animal medicine (Flynn, 1973) and pinworm infections in laboratory colonies remain common to this day (Michels et al., 2006; Baker, 2007). Pinworms can be surprisingly difficult to eliminate from the animal facility (Taffs, 1976; Baker, 2007).

Peptidases (proteolytic enzymes or proteases) are enzymes which hydrolyze peptide bonds. They catalyze the cleavage of amide linkages in macromolecular proteins and oligomeric peptides (Sajid and McKerrow, 2002). Peptidases play essential roles in cellular, developmental and digestive processes, blood coagulation, inflammation, wound healing and hormone processing (Williamson et al., 2003). For this reason it is not surprising that peptidases play a critical role in the pathogenesis of parasitic diseases (McKerrow et al., 1990, 2006). Many helminth parasites release peptidases to accomplish some of the tasks imposed by a parasitic lifestyle, including tissue penetration, digestion of host tissue for nutrition and evasion of host immune responses (Tort et al., 1999). Peptidases are extensively studied, especially in helminth parasites with veterinary (Young et al., 1995; Hadaś and Stankiewicz, 1997a,b; Geldhof et al., 2002; Redmond et al., 2006), or humane importance (McKerrow et al., 1990; Brinkworth et al., 2001; Sajid et al., 2003) but there is no report about these important enzymes in pinworms.

This work focuses on peptidases of pinworms *S. muris* and *P. ambiguus*, and characterizes these enzymes according to their substrate specificity and inhibitor sensitivity using substrate polyacrylamide gel electrophoresis.

2. Material and methods

2.1. Collection of parasites

Adult male laboratory rats (Wistar) and rabbits (New Zealand White), naturally infected by pinworms bred at the Institute of Physiology, Academy of Sciences of the Czech Republic, were examined antemortem coprologically or by Cellophane Tape Test for pinworm infection. Positive animals were slaughtered, their cecum and colon were removed, placed in a glass bottle containing

* Corresponding author.
E-mail address: vadlejch@af.czu.cz (J. Vadlejch).

0014-4894/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2010.04.018

saline and the contents were decanted several times. Adult worms (males and females) collected from cecum and colon contents were rinsed in phosphate-buffered saline (PBS) pH 7 and microscopically determined according to their morphological signs as *S. muris* and *P. ambiguus*, respectively.

2.2. Parasite cultures

Adults of appropriate pinworms were maintained for 20 h at 37 °C in a sterile RPMI 1640 medium supplemented with penicillin (1000 U/ml), gentamycin (1 μg/ml), streptomycin (5 mg/ml) and L-glutamine (10 mM) (all purchased from Sigma–Aldrich). The culture supernatants were concentrated at 4 °C using a Microcon YM-10 centrifugal filter device (Millipore). Culture fluid containing in vitro released products of nematodes are referred to as IVR. Protein concentration in IVR products was measured by the Bicinchoninic Acid Protein Assay (Sigma–Aldrich) and products were stored prior to analysis in a deepfreezer box at minus 70 °C.

2.3. Substrate gel analysis

For the investigation of IVR protein profile IVR products were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) under non-reducing conditions (Hoefer miniVE, Amersham Biosciences) in 10% gels. All stock solutions and techniques were made according to Bollag and Edelstein (1991). Before loading on the gel, samples were diluted in Laemmli sample buffer without 2-mercaptoethanol. IVR proteins (20 μg per well) were applied to the gel and fractionated under non-reducing conditions. Electrophoresis was performed initially for 30 min at 90 V and then for 80 min at 150 V using an ice-cold electrode buffer. Once the run was finished, gels were washed in three changes of 2.5% Triton X-100 over a 30 min period to remove the SDS and then incubated in 0.1 M citrate–phosphate buffer (pH 7) overnight at 37 °C. Proteins were visualized by Coomassie Brilliant Blue R-250 (Sigma–Aldrich).

Peptidase activity of IVR samples was assayed by zymography in 10% gels, where various protein substrates as gelatin, bovine albumin, bovine mucin and hemoglobin (Sigma–Aldrich) were incorporated at a final concentration of 1 mg/ml. Samples were mixed with a non-reducing sample buffer and loaded onto the gel (20 μg protein per well). SDS–PAGE was performed on ice by the same process as the protein profile above. After electrophoresis, the gels were sliced prior to overnight incubation in the appropriate buffer – 0.1 M citrate–phosphate buffer (pH 3, 5, and 7) and in 0.1 M Tris–HCl buffer respectively (pH 9). The zone of proteolysis was visualized by Coomassie Blue staining.

2.4. Inhibitor sensitivity analysis

The effect of specific peptidase inhibitors (Sigma–Aldrich) on enzyme activity was determined by pre-incubating samples with an appropriate inhibitor for 30 min at the following final concentration prior to loading onto the gels: 10 μM trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butan (E-64 for cysteine peptidases), 10 mM ethylenediamino-tetra-acetic acid (EDTA for metallo-peptidases), 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF for serine peptidases) and 1 μM (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid (pepstatin A for aspartyl peptidases). After electrophoresis, gels were sliced and washed several times thoroughly in 2.5% Triton X-100, and incubated overnight in a buffer containing the appropriate specific inhibitor. Reduced intensity of proteolysis or their total disappearance in the presence of a specific inhibitor was visualized by Coomassie Blue counter-staining.

3. Results

3.1. Proteolytic assays

Protein profiles of *S. muris* and *P. ambiguus* IVR products are shown in Fig. 1. Zones of proteolysis were observed in IVR peptidases of both pinworms on gelatin and mucin substrate gels. Proteolytic activity on albumin and hemoglobin gels was observed in neither IVR peptidases of *S. muris*, nor in that of *P. ambiguus*. Two proteolytic zones were observed on *Syphacia* gelatine gel, one strong band approximately at 25 kDa and the second, less obvious band, with a molecular weight (MW) of 30 kDa. Both these bands were observed at only pH 9 (Fig. 2). Very weak proteolytic activity on *Syphacia* mucin gel was seen at a neutral and alkaline pH levels. At pH 9 zones with a broad MW – 45, 60 and 110 kDa and only one 42 kDa band at pH 7 were observed (Fig. 2). Very strong proteolytic activity was visible in both *Passalurus* gelatin and mucin substrate gels. Very strong proteolytic zones were marked in gelatine gel (Fig. 3) in all tested pH levels (3, 5, 7, and 9) with a broad molecular weight ranging from 20 kDa to high MW (>200 kDa). Strong zones between 23 and 50 kDa were evident at pH 3 on mucin gel and four less strong bands – one weak 23 kDa band, a double band with MW between 25 and 30 kDa and one less obvious band with low MW (18 kDa) were observed on the same gel. All these observed zones were visible only at pH 5. No activity was observed on *Passalurus* mucin gel at pH 7 and 9 (Fig. 3).

3.2. Inhibitor sensitivity

Proteolytic zones on *Syphacia* gelatin gel at approximately 25 and 30 kDa were completely inhibited at pH 9 by AEBSF, a specific inhibitor of serine peptidases (Fig. 4). Three bands visible on the *Syphacia* mucin substrate gel were completely inhibited by AEBSF at pH 9 and their activity was reduced by EDTA, a specific inhibitor of metallo-peptidases, at the same pH level (Fig. 4). At pH 5, zones of proteolysis with low MW levels (<30 kDa) in *Passalurus* gelatin gel were completely abolished by AEBSF, and the zones with MW levels above 30 kDa were inhibited by specific inhibitor of aspartyl peptidases–pepstatin A. About 85 kDa strong band and less evident 65 and 70 kDa bands were inhibited at pH 9 concurrently by AEBSF and pepstatin A. The activity of the zone with MW 80 kDa was reduced only by pepstatin A (Fig. 5). The other proteolytic zones at pH 3, 7 and 9 with MW levels above 30 kDa were inhibited in

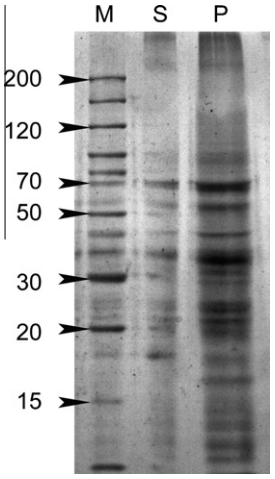

Fig. 1. Protein profile of *S. muris* (S) and *P. ambiguus* (P) IVR products. Molecular weight marker (M) is indicated in the figure on the left in kilodaltons. The 20 μg of IVR proteins from adult pinworms was subjected to 10% SDS–PAGE under non-reducing conditions and stained by Coomassie Blue.

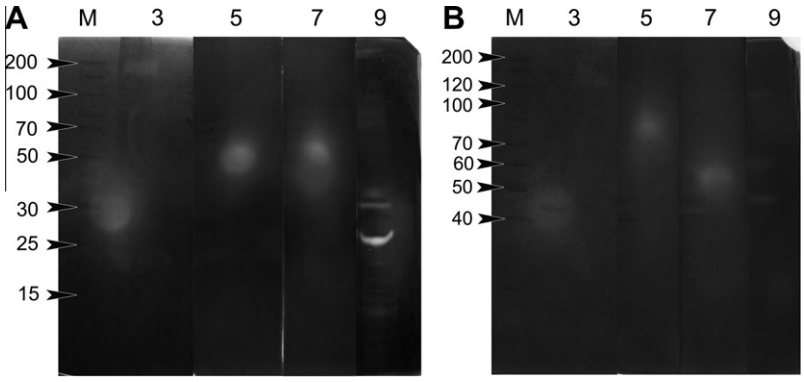

Fig. 2. Substrate gelatin and mucin gel of *S. muris* IVR peptidases at various pH. About 20 μg of IVR protein was loaded on 10% gel with incorporated protein (A – gelatin and B – mucin) and electrophoresed under non-reducing conditions. Gel strips were incubated in buffers of varying pH. Lane 3 – 0.1 M citrate–phosphate buffer (pH 3), lane 5 – 0.1 M citrate–phosphate buffer (pH 5), lane 7 – 0.1 M citrate–phosphate buffer (pH 7), and lane 9 – 0.1 M Tris–HCl buffer (pH 9). Zones of proteolysis are clear bands against a blue background. The position of the protein size marker (M – MW marker by Fermentas) is indicated in the figure in kDa. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

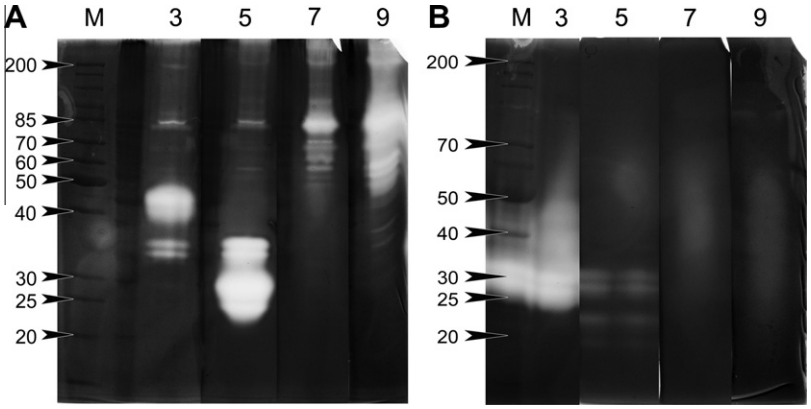

Fig. 3. Gelatin and mucin gel of *P. ambiguus* IVR peptidases. IVR protein (20 μg per well) was loaded on the gel containing protein substrate (A – gelatin and B – mucin) and was subjected to non-reducing SDS–PAGE. Gel strips were incubated at pH 3 (lane 3), pH 5 (lane 5), pH 7 (lane 7) and pH 9 (lane 9). Proteolytic zones are clear bands against a blue background. MW marker (M) is indicated in the figure in kDa.

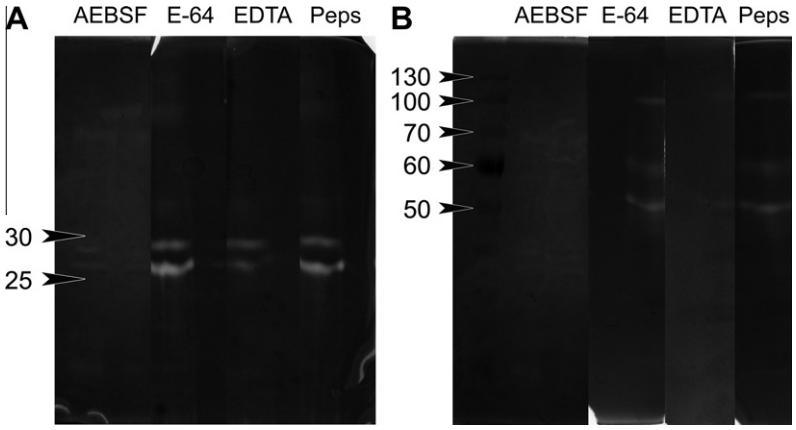

Fig. 4. The effect of inhibitors on *S. muris* IVR peptidases on gelatin (A) and mucin (B) substrate gel. About 20 μg of IVR protein was loaded on the gel, electrophoresed and gel strips were incubated in 0.1 M Tris–HCl buffer (pH 9) containing specific inhibitors – 1 mM AEBSF (serine peptidases), 10 μM E-64 (cysteine peptidases), 10 mM EDTA (metallo-peptidases) and 1 μM pepstatin A (aspartyl peptidases). MW standards are indicated in the figure in kDa.

the same manner as the zones above (data not shown). Strong zones of proteolysis at pH 3 on *Passalurus* mucin gel between 25 and 50 kDa were abolished only by pepstatin A (Fig. 5), bands at pH 5 under 30 kDa were partially reduced by E-64 (not shown).

4. Discussion

One of the crucial adaptations for parasitism is the ability to secrete products that modify the host environment to maximize fit-

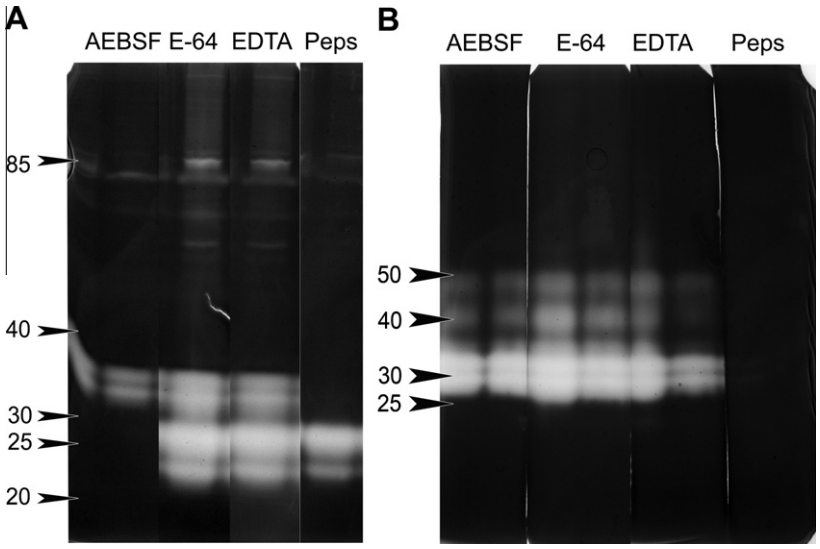

Fig. 5. Gelatin and mucin substrate gel with *P. ambiguus* IVR peptidases incubated with specific inhibitors. IVR protein (20 μg in each lane) was subjected to non-reducing SDS–PAGE, gel strips were incubated in 0.1 M citrate–phosphate buffer – gelatine gel (A) in pH 5 and mucin gel (B) in pH 3, all with appropriate specific inhibitor – AEBSF, E-64, EDTA, pepstatin A. The position of the size marker is indicated on the left in kDa.

ness of the parasite (Thompson and Geary, 2002). These excretory/secretory products (or IVR products) contain bioactive molecules as various proteins and enzymes, without their existence it would be definitely impossible for parasites to survive. One of the most important parts of IVR products are peptidases used by helminth parasites especially for invasion, feeding, digestion and for evasion of the host immune system. For this reason IVR products and peptidases are extensively studied (reviewed by Tort et al., 1999; Sajid and McKerrow, 2002; Dzik, 2006).

In this experiment, we studied IVR peptidases of two common pinworms *S. muris* and *P. ambiguus* and we used a variety of protein substrates as gelatin, bovine albumin, bovine mucin and hemoglobin. Gelatin is a water-soluble skleroprotein that derives by boiling insoluble collagen. Collagen is chemically subdivided into numerous types and the predominant ubiquitous type is the type I occurring especially in skin, bone, tendom, ligaments, and in principle in the fascia and connective tissue of all organs (Samuelson, 2007). At alkaline pH levels *Syphacia* IVR peptidases showed marked activity against gelatin, and *Passalurus* IVR peptidases were active on the same substrate in a broad scale from acidic to alkaline pH levels (3–9). IVR peptidases of *Syphacia* with gelatinolytic activity were completely inhibited at pH 9 by AEBSF, which is known to inhibit serine peptidases. We can assume that these observed serine peptidases with MW 25 and 30 kDa are probably used by migration of worms through the cecum and colon, which is alkaline and mostly varies from pH 6 to 8. *Passalurus* gelatin substrate gel revealed a number of IVR peptidases with various molecular sizes which are active in a broad pH scale. At alkaline pH levels serine peptidases with MW levels under 30 kDa were detected and bands between 30 and 35 kDa were reduced by a specific inhibitor of aspartyl peptidases pepstatin A. Some high molecular weight proteolytic zones were abolished by AEBSF and pepstatin A concurrently, but their further identification remains obscure. We can presume that detected serine and aspartyl peptidases can be utilized by pinworm females by moving in the perianal area, where they lay eggs. The activity of these enzymes at acidic pH is in accordance with the pH level of the skin (pH 4.5–5.5). It is known, that worms can change the pH levels of their microenvironment and this can be radically different from the pH levels under normal physiological conditions. Dawes (1974) indicated that in a relatively short time helminth parasites can acidify the pH of a medium by about 1 pH unit, and Maki and Yanagisawa (1986) suggests a difference of up to 3 pH units. These findings can explain the peptidase activity at very acidic pH 3. Aspartyl *Passalurus* peptidases active at alkalic pH can, similarly to IVR peptidases of *Syphacia*, serve passage through the large intestine environment.

Gelatin is considered as a general peptidase substrate and is used in many similar studies as the only option (de Cock et al., 1993; Young et al., 1995; MacLennan et al., 1997). For a better understanding of the purpose of pinworm IVR peptidases we incorporated the other substrates into the gels. Mucins are major components of the mucus that cover the surface of cell lining the respiratory, digestive and urogenital tracts and these glycoproteins are a part of a defensive system at mucosal surface of the gastrointestinal tract. They protect epithelial cells from infection, dehydratation and physical and chemical injury and they aid the passage of materials through the tract (Perez-Vilar and Hill, 1999; Corfield et al., 2001). *S. muris* release weak peptidases with mucinolytic activity at alkaline pH and their activity is inhibited by AEBSF and by EDTA, but classification of these peptidases remains unclear. *Syphacia* is rarely attached to the mucous membrane and stays in the contents of the large intestine, but there are some reports about health problems such as mucoid enteritis, which is thought to be associated with heavy worm burdens (Harwell and Boyd, 1968; Jacobson and Reed, 1974). So it is possible that in severe infections with heavy worm burden some of these worms are attached to the mucous membrane and use mucins for feeding. For this purpose pinworms can use peptidases which are active against mucin at alkaline pH (pH 7 and 9). Aspartyl and cysteine peptidases which are active against mucin at acidic pH levels are components of IVR products of *P. ambiguus*. These enzymes could serve pinworm females in their return to the colon from the acidic environment of the posterior parts of the rectum. They can use aspartyl and cysteine peptidases for degrading mucin and it could be an important nutrient for this parasite. Substrate gels with incorporated blood proteins such as albumin and hemoglobin were without any visible zones of proteolysis in both IVR products of *Syphacia* and *Passalurus* and these results are in accordance with the conclusion that none of the pinworms is a blood-feeder.

This study provides a firsthand look into the world of pinworms in vitro maintained peptidases. Of course, this is merely an early study about this topic and it is necessary to verify obtained results

because the purpose of the determined released peptidases is only speculative and remains unclear. However, it is surprising that pinworms, which are generally considered to be a relatively non-pathogenic parasite, release such potent peptidases which are active in a broad pH spectrum. The next very interesting thing is that IVR peptidases of *S. muris*, when compared to *Passalurus* IVR peptidases, are very sensitive and all analyses must be maintained on ice otherwise their activity is completely destroyed and it is impossible to detect them by SDS–PAGE. Further research on this topic could look at the IVR peptidases influence on immune response of the host, or at the IVR peptidases of pinworms larvae because it was ascertained that 4th-stage larvae of *P. ambiguus* can, under certain conditions, penetrate the mucous membrane. All new informations about peptidases of pinworms can lead to a better understanding of the biology, epidemiology and possible pathology of these common, but to this date, insufficiently recognized helminth parasites.

Acknowledgment

This study was supported by the Research Project of the Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, No. MSM 6046070901. All experiments conducted with laboratory animals comply with the current laws of the country in which they were performed.

References

Baker, D.G., 2007. Flynńs Parasites of Laboratory Animals. Blackwell Publishing, Iowa.

Bollag, D.M., Edelstein, S.J., 1991. Protein Methods. Wiley-Lis, Inc., New York.

Brinkworth, R.I., Prociv, P., Loukas, A., Brindley, P.J., 2001. Hemoglobin-degrading, aspartic proteases of blood-feeding parasites – substrate specificity revealed by homology models. Journal of Biological Chemistry 276, 38844–38851.

Corfield, A.P., Carroll, D., Myerscough, N., Probert, Ch.J.S., 2001. Mucins in the gastrointestinal tract in health and disease. Frontiers in Bioscience 6, 1321–1357.

Dawes, B., 1974. Ecological and physiological aspects of helminth–host interactions in the mammalian gastrointestinal canal. Advances in Parasitology 12, 183–278.

De Cock, H., Knox, D.P., Claerebout, E., De Graaf, D.C., 1993. Partial characterization proteolytic enzymes in different developmental stages of *Ostertagia ostertagi*. Journal of Helminthology 67, 271–278.

Dzik, J.M., 2006. Molecules released by helminth parasites involved in host colonization. Acta Biochimica Polonica 53, 33–64.

Flynn, R.J., 1973. Parasites of Laboratory Animals. Iowa State University Press, Ames.

Geldhof, P., Claerebout, E., Knox, D., Vercauteren, I., Looszova, A., Vercruysse, J., 2002. Vaccination of calves against *Ostertagia ostertagi* with cysteine proteinase enriched protein fractions. Parasite Immunology 24, 263–270.

Hadaś, E., Stankiewicz, M., 1997a. Proteolytic enzyme of infective larvae and adults of *Trichostrongylus colubriformis* and *Haemonchus contortus*. Parasitology Research 83, 47–51.

Hadaś, E., Stankiewicz, M., 1997b. The isolation, partial purification and some characteristics of proteasomes from *Trichostrongylus colubriformis* larvae. Acta Parasitologica 42, 121–123.

Harwell, J.F., Boyd, D.D., 1968. Naturally occurring oxyuriasis in mice. Journal of the American Veterinary Medical Association 153, 950–953.

Jacobson, R.H., Reed, N.D., 1974. The thymus dependency of resistance to pinworm infection in mice. The Journal of Parasitology 60, 976–979.

Maclennan, K., Gallagher, M.P., Knox, D.P., 1997. Stage-specific serine and metalloproteinase release by adult and larval *Trichostrongylus vitrinus*. International Journal for Parasitology 27, 1031–1036.

Maki, J., Yanagisawa, T., 1986. Demonstration of carboxyl and thiol protease activities in adult *Schistosoma mansoni*, *Dirofilaria immitis*, *Angiostrongylus cantonensis* and *Ascaris suum*. Journal of Helminthology 60, 31–37.

McKerrow, J.H., Brindley, P., Brown, M., Gam, A.A., Stauton, C., Neva, F.A., 1990. *Strongyloides stercoralis*: identification of the protease that facilitates penetration of skin by the infective larvae. Experimental Parasitology 70, 134–143.

McKerrow, J.H., Caffrey, C., Kelly, B., Loke, P., Sajid, M., 2006. Proteases in parasitic diseases. Annual review of pathology 1, 497–536.

Michels, C., Goyal, P., Nieuwenhuizen, N., Brombacher, F., 2006. Infection with *Syphacia obvelata* (pinworm) induces protective TH2 immune responses and influences ovalbumin-induced allergic reactions. Infection and Immunity 74, 5926–5932.

Owen, D., 1972. Common Parasites of Laboratory Rodents and Lagomorphs. Laboratory Animals Centre Handbook No. 1. Medical Research Council.

Perez-Vilar, J., Hill, R.L., 1999. The structure and assembly of secreted mucins. Journal of Biological Chemistry 274, 31751–31754.

Redmond, D.L., Smith, S.K., Halliday, A., Smith, W.D., Jackson, F., Knox, D.P., Matthews, J.B., 2006. An immunogenic cathepsin F secreted by the parasitic stages of *Teladorsagia circumcincta*. International Journal for Parasitology 36, 277–286.

Rinaldi, L., Russo, T., Schioppi, M., Pennacchio, S., Cringoli, G., 2007. *Passalurus ambiguus*: new insights into copromicroscopic diagnosis and circadian rhythm of egg excretion. Parasitology Research 101, 557–561.

Rudolphi, C.A., 1819. Entozoorum synopsis cui accedunt mantissima duplex et indices locupletissima. Sumtibus Augusti Rücker, Berolini. 811 pp.

Sajid, M., McKerrow, J.H., 2002. Cysteine proteases of parasitic organisms. Molecular and Biochemical Parasitology 120, 1–21.

Sajid, M., McKerrow, J.H., Hansell, E., Mathieu, M.A., Lucas, K.D., Hsieh, I., Greenbaum, D., Bogyo, M., Salter, J.P., Lim, K.C., Franklin, C., Kim, J.H., Caffrey, C.R., 2003. Functional expression and characterization of *Schistosoma mansoni* cathepsin B and its trans-activation by an endogenous asparaginyl endopeptidase. Molecular and Biochemical Parasitology 131, 65–75.

Samuelson, D.A., 2007. Textbook of Veterinary Histology. Saunders Elsevier, Saint Louis. 380 pp.

Stahl, W.B., 1963. Studies on the life cycle of *Syphacia muris*, the rat pinworm. The Keio Journal of Medicine 12, 55–60.

Taffs, L.F., 1976. Pinworm infections in laboratory rodents: a review. The International Journal of Laboratory Animal Science and Welfare 10, 1–13.

Thompson, D.P., Geary, T.G., 2002. Excretion/secretion, ionic and osmotic regulation. In: Lee, D. (Ed.), The Biology of Nematodes. CRC Press, New York.

Tort, J., Brindley, P.J., Knox, D., Wolfe, K.H., Dalton, J.P., 1999. Proteinases and associated genes of parasitic helminths. Advances in Parasitology 43, 161–266.

Williamson, A.L., Brindley, P.J., Knox, D.P., Hotez, P.J., Loukas, A., 2003. Digestive proteases of blood-feeding nematodes. Trends in Parasitology 19, 417–423.

Yamaguti, S., 1941. Studies on the helminth fauna of Japan, Part 13: Mammalian nematodes. Japanese Journal of Zoology 9, 409–439.

Young, C.J., McKeand, J.B., Knox, D.P., 1995. Proteinases released in vitro by the parasitic stages of *Teladorsagia circumcincta*, an ovine abomasal parasite. Parasitology 110, 465–471.