Table I: Tissue Distribution of Collagenase and LAP in *S. cervi*[a].

Tissues	Relative Staining Intensity	
	Collagenase	Leucine Aminopeptidase (LAP)
Epiuticle	++++	++++
Cuticle	++++	-/+
Syncytial hypodermis	++++	+
Longitudinal muscle layers	++	++++
Endodermis	+++	++++
Intestine	+++	++++
Ovary growth zone	++	**NS**
Ovary germinal zone	++	++
Uterine wall	+++	++++
Eggs		
(a) Immature	-	++
(b) Mature	+++	+++
Embryo	+++	+++
Microfilariae (mf)	++	++++

[a]++++: Strongly positive; +++: Positive; +++: Moderately positive; ++: Positive; + or +/-: Faintly positive: -: Negative; NS: Not Studied

Immunohistochemistry (IHC) of collagenase

Thin sections (5 μm) of the paraffin embedded tissues of *S. cervi* aimed for IHC of collagenase were cut using a microtome and spread over 1% gelatin coated slides which were covered with distilled water and warmed at 55–60°C. The immunohistochemistry was performed as described elsewhere [27]. Briefly, sections were deparaffinised with three changes of xylene and rehydrated through graded alcohols and finally with water. The endogenous peroxidase activity was inactivated by incubating these sections in 3% H_2O_2 in methanol for 30 min at room temperature. Next, the slides were rinsed with three changes of PBS and the sections were incubated with 2% normal rabbit blocking serum for 1 h. The excess blocking serum was shed off and the sections were further incubated overnight at 4°C with primary antibody obtained from jirds (*Meriones unguilatus*) immunized with purified collagenase (1:200 dilutions). The sections were rinsed thrice with PBS and incubated for 1 h with horseradish peroxidase conjugated secondary antibody (1:2500 dilutions) at room temperature. The antigen-antibody complex was then visualized by incubating the sections with 3, 3'-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris-HCl, pH 7.6, and 0.015% H_2O_2) in dark for 15–30 min. The slides were then counterstained with Meyer's hematoxylin and treated through graded alcohols and xylene, and mounted with DPX. Sections exposed to preimmune jirds sera were always included as negative controls. Permanent slides were examined using a light microscope (Nikon Eclipse E800, Nikon, Tokyo, Japan) and photographed with Nikon digital Camera DXM 1200.

Histochemical staining of leucine aminopeptidase (LAP)

The parasite tissues fixed in formol-calcium were cut into thin sections (5 μm) and spread over gelatin coated slides as described above for collagenase. The sections were deparaffinised with xylene and rehydrated through graded alcohols and water. LAP histochemical staining was performed according to the method described elsewhere [28]. Briefly, the rehydrated sections were incubated with the incubating solution [L-Leu-β-naphthylamide 0.5 mM, Potassium cyanide (0.00065%), sodium chloride (0.34%) and Fast Blue B salt (0.05%)] in a moist dark chamber for 2 h. Next, the slides were rinsed consecutively in 0.85% saline, 0.1 M copper sulphate and again with saline for 2 min each. The sections were then counterstained with 2% methyl green for 3 min and rinsed in distilled water. Finally the counterstained sections were treated through the graded alcohols, xylene and mounted in DPX for permanent slide preparation. Sections for negative controls were incubated in the incubating medium without the substrate. The slides were observed under a light microscope (Nikon Eclipse E800, Nikon, Tokyo, Japan) and photographed with Nikon digital Camera DXM 1200.

Results

Distribution of collagenase in S. cervi

We examined the localization of 175kDa collagenase in various tissues of adult *S. cervi* worms including eggs and microfilariae using IHC and assessed the immunostaining from at least five sections obtained from different body regions of the three separate worms. The results of immunostaining are summarized in Table 1.

The appearance of dark brown color of DAB was considered as the basis for evaluation of positive staining in the parasite tissues. Very strong immunostaining was observed in the epicuticle and cuticle followed by syncytial hypodermis. Good staining was observed in longitudinal muscle layers, endodermis, intestinal wall, uterine wall, ovary (growth and germinal zones), and periphery of mature eggs and growing embryo inside the uterus. The intensity of enzyme staining was found to be gradually increased from immature ovary to fully-grown ovary. The staining in mf was, however, found to be localized in certain secretary pores and body cavity when viewed under higher magnification (Fig 1B–G). Faint staining was observed in the innervation process beneath the body wall. No staining was observed in the spaces between longitudinal muscle layers and the tissue inside the uterus. No such staining was observed in control sections in which preimmune jirds sera were used as primary antibody (Fig 1A).