

Characterization of Antigenic Property of Adult *Spirocerca lupi* Collected from Esophageal Nodules in Dogs

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ABSTRACT

Spirocerca lupi, the esophageal nematode of dog, causes a potentially fatal disease in domestic dogs. Immunological techniques can identify the parasite proteins which elicit an immune response. However, the antigenic properties of *S. lupi* adult worms have not been fully understood. The immuno-reactivity of the naturally infected dog sera with the *S. lupi* somatic antigens showed 7 prominent immunoreactive bands of distinct sizes at 199, 148, 100, 87, 49, 16, and 12 kDa, whereas 8 prominent bands at 230, 200, 100, 60, 49, 29, 16, and 12 kDa against hyper immune serum raised in the rabbits. Common antigen bands for both types of serum were observed at 100, 49, 16 and 12 kDa. Intriguingly, the current study was able to indicate the detailed antigenic profile with specific molecular moieties which might be a manifesto for future investigation. Identified specific antigens merit further analysis as potential tools for the evaluation of treatment success and prognosis of spirocercosis, and development of a sensitive and specific diagnostic test for early detection of *S. lupi* in infected dogs.

Keywords: Dog, Somatic antigen, *Spirocerca lupi*.

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INTRODUCTION

Spirocerca lupi is a nematode of worldwide distribution but is commonly found in tropical and subtropical areas (McGavin & Zachary, 2007; Traversa *et al.*, 2008). Spirocerciasis is usually subclinical, but the chronic disease states associated with *S. lupi* infection in dogs causes aortic scarring with aneurysm formation and thoracic vertebra spondylitis and caudal esophageal nodule formation. The typical clinical signs associated with spirocercosis are related to the presence of esophageal nodules and include regurgitation, vomiting, dysphagia and weight loss, together with non-specific signs like pyrexia (Dvir *et al.*, 2001; Lobetti, 2000; Mazaki-Tovi *et al.*, 2002). About 25% of esophageal nodules associated with spirocercosis progress to a pre-neoplastic and finally into osteosarcoma, fibrosarcoma or undifferentiated sarcomas (Dvir *et al.*, 2001). However, sudden death has also been reported secondary to aneurysmal rupture of the aorta (Van der Merwe *et al.*, 2007). Prevalence values of spirocercosis depend mainly on the density of the canine population and the degree of contact between definitive, intermediate and transport hosts.

Various diagnostic techniques are available for identifying spirocercosis in dogs with certain limitations. Fecal flotation tests may give false-negative results if there are single-sex, pre-patent, or senile infections and variation in egg shedding (Dvir *et al.*, 2001). Endoscopy is a sensitive and reliable diagnostic technique but requires general anesthesia and only detects nodules and adult worms that protrude into the lumen of the esophagus (Markovics & Medinski 1996). Though the radiographic features of clinical spirocercosis have been described (Dvir *et al.*, 2001), there is no data on the sensitivity

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and specificity of radiography for diagnosing infections. However, due to the chronic nature of spirocercosis, dogs are usually diagnosed in advanced disease stages, which are associated with severe detectable clinical signs (Traversa *et al.*, 2008).

In the absence of parasitological evidence of infection, immunological methods are required for its diagnosis. Immunological techniques can identify the parasite proteins which elicit an immune response. However, the antigenic properties of *S. lupi* adult worms have not been fully

understood. The present study aimed to characterize the somatic antigens of adult-*Spirocerca lupi* against the serum of naturally infected dogs and hyperimmune serum raised in rabbit. To our knowledge, this study was the first attempt in the identification and characterization of somatic antigenic property of *S. lupi* adults obtained from esophageal nodules of naturally infected dogs.

MATERIALS AND METHODS

This experiment was carried out in accordance with the guidelines for animal experimentation of the Faculty of Veterinary Medicine and Animal Sciences, Peradeniya University, Sri Lanka.

Somatic antigens of *S. lupi* were prepared according to the method described by Kupai, K. (2010). Briefly, the freshly isolated adult *S. lupi* worms from esophageal nodule (Figure 1A) from 7 necropsied dogs were washed three times thoroughly with phosphate buffer saline (PBS) before extraction of protein. Worm tissue was mixed with PBS in 1:4 ratio (30 mg tissue + 120 μ l PBS). The sample was homogenized for 30 seconds with pestle homogenizer in ice. The homogenized samples were centrifuged, supernatant was collected, filtered through millipore filters (0.2 microns) and stored at -20 °C until further use. Protein estimation was done by using bicinchoninic acid protein assay kit (Sigma-Aldrich Inc., St. Louis, MO, USA). Characterization of somatic soluble proteins was carried out by Sodium dodecyl sulphate-electrophoresis (SDS-PAGE). The samples were prepared by mixing antigen with SDS-PAGE sample buffer 1:2 ratio in eppendorf tubes and heating at 100°C for 10 minutes in water bath. Electrode buffer pH 8.3 was filled in to the upper and lower buffer chamber. A 20 μ l of each sample was loaded in to each well. Gel was run for 15min at 100 mv and 35 minutes at 200 mv. After electrophoresis, the stacking gel was removed and separating gel was stained. The separating gel was stained for one hour with 0.1% Coomassie Brilliant Blue R-250 stain in 40% Methanol and 10% Acetic acid and destained with the same solution sans Coomassie Brilliant Blue until clear band appeared (Sigma Chemicals, UK).

Hyper-immune serum was raised by using 6 months old male New Zealand rabbits (n=3) (body weight 1.7 \pm 0.35 kg) purchased from the Medical Research Institute (Colombo, Sri Lanka). The rabbits were housed (1 per cage) and fed with grower pellets (Musajees Ltd, Colombo, Sri Lanka) and water was available at libitum. The rabbits were injected with *S. lupi* somatic antigen at the dose rate of 200 μ g per animal as a deep intramuscular injection. The somatic soluble antigen of adult *S. lupi* was emulsified in an equal volume of Freund's complete adjuvant (0.5 ml) (Sigma) and used for primary immunization. Booster doses were given along with Freund's incomplete adjuvant (Sigma) twice a week. Rabbits were bled after 10 days from the primary injection and 10 mL of blood sample was collected from marginal ear vein of each rabbit

and assayed for antibody titer by dot blot and agarose gel precipitation test. Usually giving a positive reaction in dot blot at dilution of 1:400 or greater is considered as a good antibody titer and this level was observed in 53 days. Western blotting was done to recognize the immunodominant polypeptides of *S. lupi* antigen according to the study of Burnette (1981) with slight modifications. Briefly, the resolved proteins were subsequently transferred to a nitrocellulose membrane. Successful transfer of the protein to the membrane was confirmed by staining the membrane with ponceau-s stain. The strips were incubated with naturally infected dogs sera and the hyperimmune sera raised in rabbit. Finally, strips were incubated with goat anti-rabbit IgG-HRP (Sigma) and with rabbit anti-dog IgG-HRP (Sigma), followed by development of blot using a diaminobenzidine color reagent system.

RESULTS AND DISCUSSION

On SDS-PAGE analysis, *S. lupi* somatic antigen revealed 14 protein bands ranging in size from 12 to 240 kDa with molecular moieties of 240, 162, 150, 124, 100, 82, 68, 49, 47, 43, 39, 24, 20, and 12 kDa (Fig. 1B). The molecular weights of protein bands were calculated by computing retardation factor (Rf) based on the standard curve drawn using a reference molecular weight marker. The specific reactivity of the somatic antigen was checked by western blotting. The naturally infected dog sera's immunoreactivity with the *S. lupi* antigens showed 7 bands at 199, 148, 100, 87, 49, 16, and 12 kDa (Fig. 1C). The immuno-reactivity of the hyperimmune serum raised in rabbits against *S. lupi* somatic antigens was observed with 8 polypeptides at 230, 200, 100, 60, 49, 29, 16, and 12 kDa (Fig. 1D). As there is no sensitive and specific diagnostic tool are needed for early detection of *S. lupi* in infected dogs, the current study was able to indicate the detailed antigenic profile with specific molecular moieties. Although, three laboratory-based diagnostic tools have been described to date for detecting *S. lupi* infection, such as technique based on concentrated sugar solution (Burnette, 1981), immunofluorescence antibody test for detecting *S. lupi* antigens, which is commercially unavailable, and its utility in clinical practice is unknown (Coskun, 1995), and a semi-nested PCR, targeting a 400 bp fragment of the mitochondrial *cox1* gene (Traversa *et al.*, 2008), their uses are limited by several factors. However, no information is available on the potential use of immunological methods for diagnosing spirocercosis in dogs.

The present study was able to identify and characterize the somatic soluble antigens of *S. lupi* adult worm by validating four common immunodominant bands at 100, 49, 16 and 12 kDa. However, additional appraisals are needed to confirm such antigens' specificity in both adult and larval stages by testing the cross-reaction with other common parasitic infections caused by nematodes. Collectively, this study might be a manifesto for future investigation of development



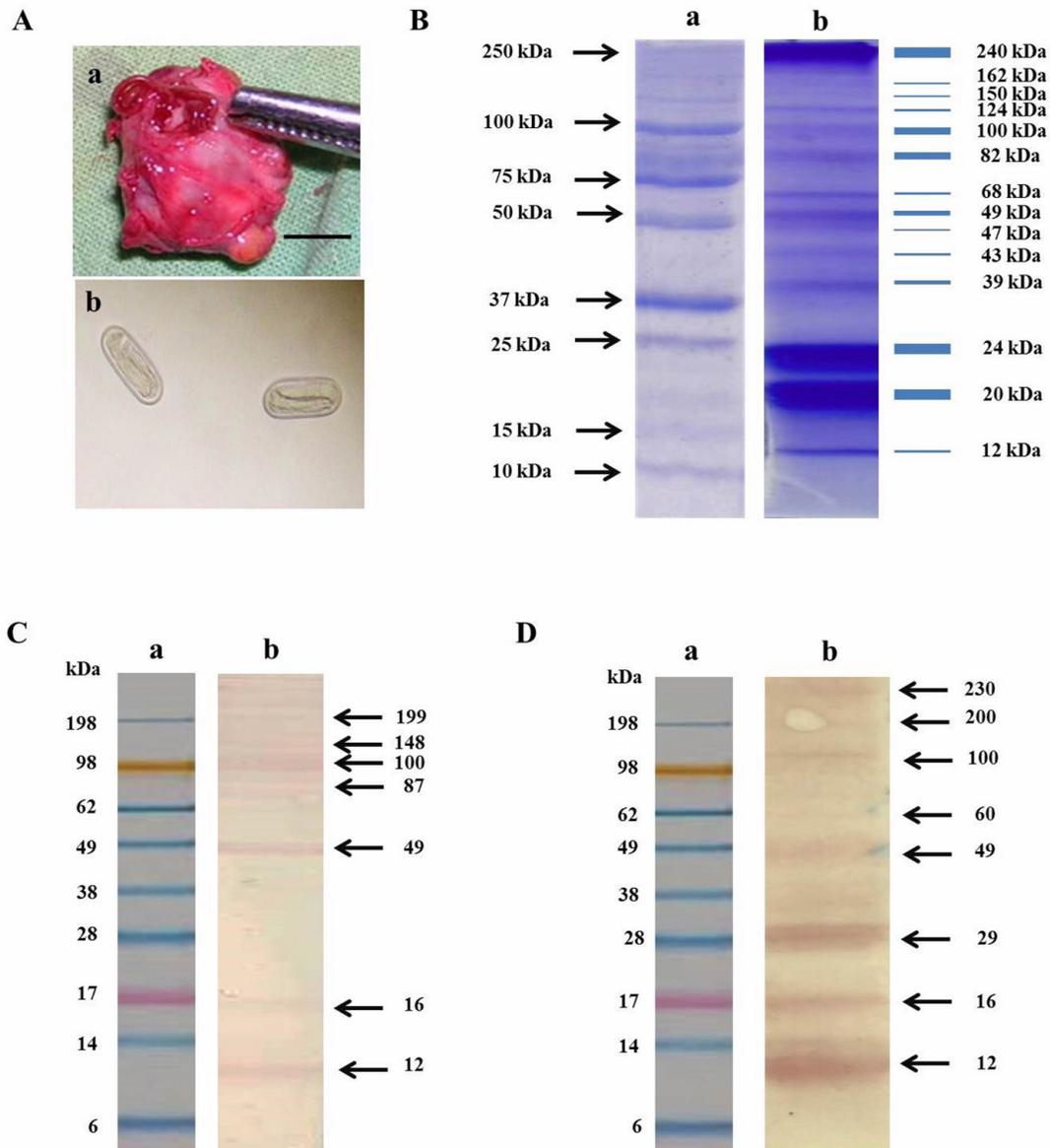


Figure 1: Shows antigenic profile of *S. lupi* adult. **(A)** a, *S. lupi* adult worm in esophageal nodule obtained from dog (scale bar-1.5 cm); b, thick-shelled larvated *S. lupi* eggs (x400) **(B)** SDS-PAGE analysis of somatic antigens of adult *S. lupi*, Lane a: protein molecular weight marker and lane b, somatic antigen of *S. lupi*. **(C)** Western blot image showing immuno-dominant polypeptides reactive to *S. lupi* infected dog serum; lane a: protein molecular weight marker and lane b: somatic antigens of adult *S. lupi*. **(D)** Western blot image showing immuno-dominant polypeptides reactive to hyper immune sera raised in rabbit against *S. lupi* somatic antigens; lane a: protein molecular weight marker and lane, b: somatic antigens of adult *S. lupi*.

of recombinant form of these antigens to standardize the routine diagnosis and carryout the epidemiological survey in canine population in endemic areas.

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