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Ascaris lumbricoides and *Ascaris suum*: A comparison of electrophoretic banding patterns of protein extracts from the reproductive organs and body wall

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ABSTRACT

Ascaris lumbricoides and *Ascaris suum* were analysed for similarities and differences in the banding patterns of proteins extracted from the body wall (BW) and reproductive organs (ROS). *Ascaris lumbricoides* and *A. suum* manifested considerable similarity in protein profile. The BW of female ascarids manifested 10 common bands, while males had 12 common bands, and of these proteins, at least five were common to both sexes. A comparison of the ROS banding profile between female and male *Ascaris lumbricodes* and *A. suum* revealed 11 and 12 common bands, respectively, of which six bands were shared. While the BW and ROS of male ascarids revealed eight shared bands, female ascarids had only two common bands. The detection of 25 kDa, 20-21 kDa and 18 kDa bands in virtually all protein samples assayed suggests their importance as housekeeping molecules and possibly valuable markers. The appreciable similarities in protein banding patterns between human and hog ascarids reflect their close genetic relationship. In view of current findings and earlier documented studies pointing to the zoonotic nature and high probability of cross-infection between human and hog species, control strategies should take into consideration the simultaneous deworming of infected persons and hogs. The likelihood of cross-infection between human and hog *Ascaris* spp. in the country can be further assessed through surveillance of human and hog ascarids, in places where backyard hog-rearing is still commonly practised. The current electrophoretic protein profiling of *Ascaris* spp. is the first of its kind in the country.

Key words: protein profile, *A. lumbricoides*, *A. suum*, Philippines, SDS-PAGE

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Introduction

Ascaris spp. are intestinal parasitic nematodes that cause ascariasis, an infection that has a cosmopolitan distribution and is highly prevalent in warm tropical countries (LORREILLE and BOUCHET, 2003; DE SILVA et al., 1997). *Ascaris lumbricoides* and *Ascaris suum* are the most important species, infecting humans and pigs respectively (CRISCIONE et al., 2007; CONDE et al., 2005; ANDERSON, 1995). In developing, as well as developed countries, ascariasis may exist more as a zoonotic infection associated with exposure to pig manure (NEJSUM et al., 2005; CONDE et al., 2005; KANORA et al., 2004; ROEPSTORFF et al., 1998). In The Philippines, *A. lumbricoides* is widespread among schoolchildren, with a prevalence ranging from 11% - 71.4% (PABLO, 1998; GARCIA, 1977). The Department of Health, Manila, Philippines reported a 66.% average prevalence of soil-transmitted helminthiasis, including *A. lumbricoides* in all regions, particularly widespread among children age 12-71 months old (VILLAVERDE et al., 2005). Despite the government's programme on intestinal parasite deworming, to this day ascariasis is still a major food/soil/water-borne infection in the country.

Human and hog ascarids bear significant morphological resemblances. Epidemiological (NEJSUM et al., 2005) and molecular (CRISCIONE et al., 2007; ABEBE et al., 2002a and 2002b) studies have provided interesting findings, suggestive of cross-infection between humans and hog *Ascaris* spp. In view of this ambiguity in species status, biological, biochemical or molecular approaches have been used to explore key characteristics useful in species differentiation. The identification of 23 essential gene markers has made possible the differentiation of human and pig ascarid populations, including the identification of hybrids (CRISCIONE et al., 2007). Also, analysis of sequences of internal transcribed spacers (ITS-1 & ITS-2) and the 5.8S genes from human and hog ascarids from different geographic locations has revealed marked similarities in nucleotides, - 100% identities in some worms - indicative of the existence of hybrid populations or allopatric variations (ZHU et al., 1999). Moreover, initial attempts by ABEBE et al. (2002a) to compare protein extracts from adult human and hog ascarids led to the detection of six major bands specific only to *A. lumbricoides*. In another study, ABEBE et al. (2002b) detected similar protein bands in the lung stage of human and hog ascarids, two of which were serologically cross-reactive.

In The Philippines, while cases of human and hog ascariasis are generally unreported, the infection is indisputably common, particularly among schoolchildren and backyard-reared hogs. In view of earlier reports suggestive of the cross-infectivity of human and hog ascarids and existence of hybrids, we compared the electrophoretic banding pattern of the Philippine *A. lumbricoides* and *A. suum*.

Materials and methods

Collection and worm segregation according to size and sex. *Ascaris suum* worms were obtained from three slaughtered hogs in an abattoir in Quezon City, Manila. Adult *A. lumbricoides* were obtained from schoolchildren living in Batangas and Manila, who had earlier been prescribed albendazole, an antihelminthic purging medicine in tablet form. The worms, inside properly labelled, re-sealable plastic bags, were placed in an icebox and sent to the research laboratory. To remove intestinal debris clinging to the worms, they were first rinsed in sterile phosphate buffered saline (PBS) solution (pH 7.4), and were then segregated according to body size and sex. The worms were washed in sterile PBS solution once again and were kept individually in properly labelled, new re-sealable plastic bags and stored in a -20 °C freezer. To prevent possible tissue degradation, the worms were kept throughout this procedure in Petri dishes placed in a cooler containing crushed ice. To prevent possible mix-ups between *A. lumbricoides* and *A. suum,* which are morphologically identical, the worms were collected at different times and were immediately segregated according to species, sex and body length. Female *A. suum* measured 156-295 mm, while the males were 114-186 mm long. Female *A. lumbricoides* were 270-340 mm long, while male worms measured 125 mm-169 mm (Fig. 1).

Separation of body wall and reproductive organs. To prevent protein degradation, individual worms placed on Petri dishes were allowed to thaw in a cooler containing crushed ice. Using a sterile scalpel and forceps, each worm was dissected by making a longitudinal antero-postero incision in the body. The white reproductive organs (ROS) that were entangled with the brown digestive tract were gently removed and the digestive tract pulled out and discarded. For each individual worm, the ROS and the body wall (BW) were placed separately in new, properly labelled, re-sealable plastic bags and stored in a -20 °C freezer, until used for homogenisation.

Homogenisation of the ascarid body wall and reproductive organs and protein extraction. Procedures for homogenisation and protein extraction were according to MAIZELS et al. (1991), with some modification. All the instruments, glassware, homogenisers, dissecting sets and micropipettes and tips, among others, and dH_20 and solutions needed for worm homogenisation, protein extraction and assay were sterilised (Hirayama Hiclave HV-85) prior to use. PBS solution and lysis buffer were stored in sterilised 100 mL reagent bottles at 4 °C. For each individual worm, the BW and ROS were separately homogenised in 1000 μL lysis buffer. To prevent protein denaturation, homogenisation was done with a glass homogeniser, partially embedded in a cooler containing crushed ice. Homogenate per sample was transferred into properly marked microcentrifuge tubes, and spun in a refrigerated centrifuge at 15,000x*g* for 15-30 min at 4 °C. The supernatant containing the proteins was stored in a -20 °C freezer, until used for protein concentration determination.

Bradford method. The Bradford method was used according to MAIZELS et al. (1991) with some modification. The standards were prepared using a mixture of bovine serum albumin (PROMEGA, USA), sterilized dH_20 and Bradford reagent (SIGMA, Coomassie Brilliant Blue G C.I. 42655). Prior to protein quantification, the supernatants were thawed in a cooler with crushed ice. A mixture of 5 µL protein sample, 95 µL dH_2O and 900µL Bradford reagent equal to 1000 µL solution was subjected to gentle vortexing. The protein concentration and absorbance of each sample was determined at 595 nm using a UV/ Vis Spectrophotometer (Daigger Genesys Spectrophotometer, GENESYS 10 UV). The protein concentration of each individual worm was recorded. Protein samples were kept in a -20 °C freezer prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE and silver staining. SDS-PAGE was carried out following the procedure of MAIZELS et al. (1991) with some modification. Protein extracts from the ROS and BW of male and female *A. suum* and *A. lumbricoides* were used. Protein samples were thawed in a cooler and then kept in a -80 °C freezer (SANYO, MDF-U52V) for 10 minutes prior to use. Seven µL of protein sample and 7 µL of treatment dye were added to a microcentrifuge tube, then centrifuged for 10 sec in a thermomixer. To separate the proteins into their monomeric form, the dyed samples were boiled for 5 to 10 min. Ten µL each of the protein samples was electrophoresed alongside another lane containing a 5µL protein marker (Broad Range PROMEGA), and run at 150V Bio-Rad Power Pac 300 (BIO-RAD) until the samples passed the stacking gel, and the voltage was increased to 300V for the proteins to separate. For band visualization, silver stain (BIO-RAD, USA) was used. The Syngene GeneTools software computer programme was used to calculate the molecular weight of protein bands. Banding pattern was then compared between the species.

Results

Protein extracts from the BW of female human and hog *Ascaris* spp. revealed 10 common distinct bands, of which the 39 kDa, 23 kDa, 20-21 and dominant 16 kDa protein were intensely stained (Fig. 2A). Similarly, extracts from the BW of male *Ascaris* spp. revealed 12 common bands (Fig. 2B), and of these bands, at least five were shared (75 kDa, 60-63 kDa, 25 kDa, 20-21 kDa, 18 kDa) with female ascarids (Fig. 2). A comparison of the ROS banding profile revealed 11 and 12 common bands in females and males respectively, of which six bands were shared (50-53 kDa, 42 kDa 25 kDa, 18 kDa, 13-15 kDa, 9-10 kDa) (Fig. 3).While the BW and ROS of male ascarids revealed eight shared bands (94-95 kDa, 60-63 kDa, 28 kDa, 25 kDa, 21 kDa, 18 kDa, 13-15 kDa, 10 kDa) (Fig. 4), females showed only two shared bands (32-35 kDa and 25 kDa) (Fig. 5). Regardless of species and sex, low molecular weight proteins such as the 25 kDa, 20-21kD and 18 kDa bands were detected in virtually all protein samples assayed in both species.

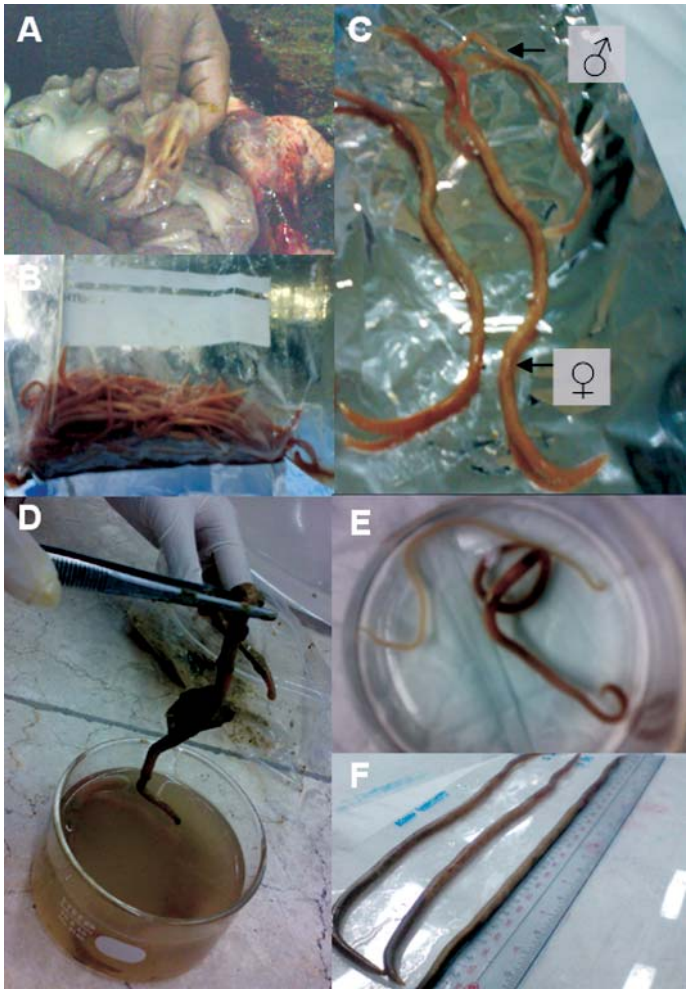

Fig. 1. *Ascaris* spp. A. Dissected infected pig's intestine; B and C *A. suum*; D, E and F *A. lumbricoides*

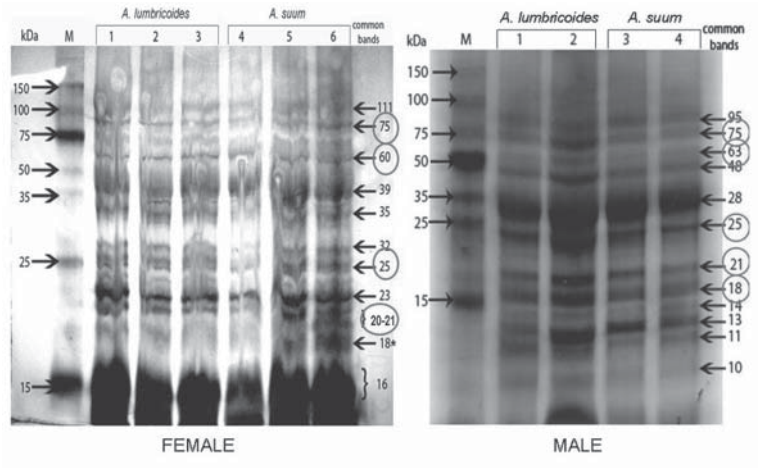

Fig. 2. Banding profiles of proteins extracted from the body wall. Comparison between female and between male *A. lumbricoides* and *A. suum*. Encircled Figs. represent shared protein bands between male and female *A. lumbricoides* and *A. suum*.

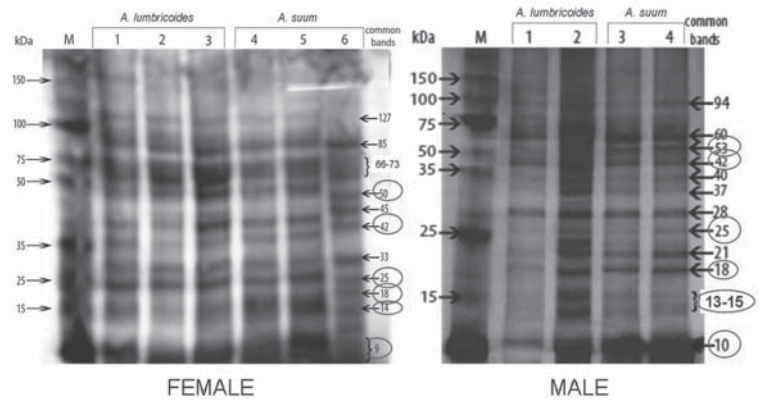

Fig. 3. Banding profiles of proteins extracted from the reproductive organs. Comparison between female and between male *A. lumbricoides* and *A. suum*. Encircled Figs. represent shared protein bands between male and female *A. lumbricoides* and *A. suum*.

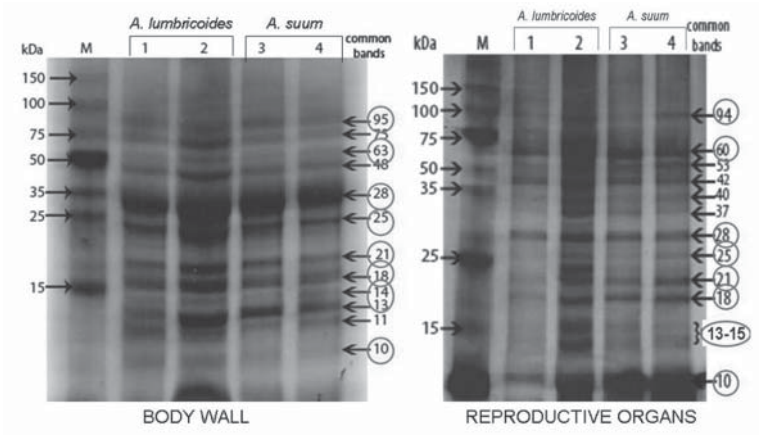

Fig. 4. Comparison of the banding profile of proteins extracted from the body wall and reproductive organs of male *A. lumbricoides* and *A. suum*. Encircled Figs. represent shared bands.

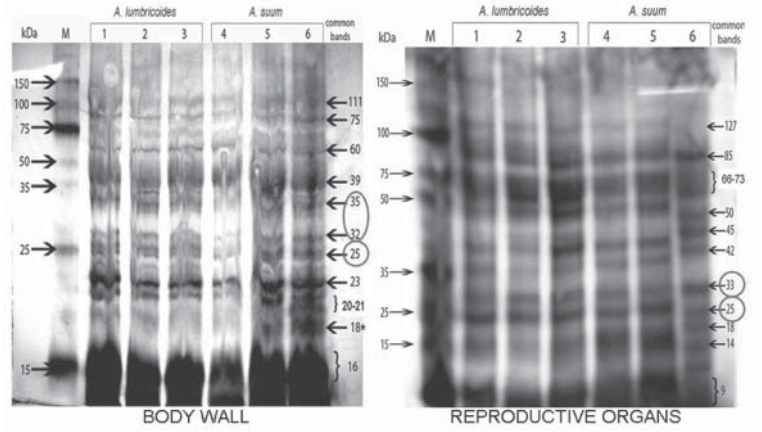

Fig. 5. Comparison of banding profile of proteins extracted from the body wall and reproductive organs of female *A. lumbricoides* and *A. suum*. Encircled Figs. represent shared bands.

Discussion

In a study on whole body extracts from adult *A. lumbricoides* and *A. suum* using two-dimensional PAGE, six dominant proteins specific to *A. lumbricoides* were detected (ABEBE et al., 2002a). In the present study, two of these bands, the 16 kDa and 13-15 kDa proteins were present in both *Ascaris* spp. The recombinant 16 kDa *A. suum* antigen was reported as protective against larval migration (TSUJI et al., 2003). Interestingly, we

found a similar 25 kDa protein invariably expressed in the BW and ROS across species and sexes, implying its importance as a housekeeping molecule and possibly, a useful marker. In a related profiling of proteins from the ascarid lung stage, the 33 kDa and 17 kDa proteins were specific to *A. lumbricoides* and *A. suum*, respectively (ABEBE et al., 2002b). The difference in the geographic location of host species, from where ascarids were collected, in the present study and those of ABEBE et al. (2002a and 2002b), and the use of two-dimensional PAGE, which allows a more effective separation of proteins compared to SDS-PAGE, may have influenced the variation in protein banding patterns between the two studies. Also, the possibility of the inclusion of host-derived proteins cannot be ruled out.

The BW and ROS of human and hog ascarids clearly showed similar electrophoretic banding profiles. While the considerable similarity in the electrophoretic banding patterns of the human and hog *Ascaris* spp. is predictable, considering the worms' striking morphologically resemblance, current data are likewise suggestive of cross-infection and species inter-breeding potential. BRYANT and FLOCKHART (1986) inferred a close similarity in enzymes between the univalens and bivalens forms of the horse *P. equorum*, as a reflection of their shared ancestry as well as their interbreeding capability.

A comparative study of gene sequences of the 5.8S rRNA and ITS-1 and ITS-2 between pig and human ascarids, collected from different geographical regions, showed six nucleotides (= 1.3%) in the ITS-1 gene specific to *A. lumbricoides*, while all parasite samples had identical 5.8S and ITS-2 gene sequences (ZHU et al., 1999). Moreover, epidemiological and gene-clustering studies generated findings suggestive of the likelihood of *A. lumbricoides* and *A. suum* hybridisation. In Denmark, for example, NEJSUM et al. (2005) reported ascariasis to be prevalent in hogs, though rare in humans, except among schoolchildren in rural areas where piggeries abound. An extension of their studies on parasite gene polymorphism indicated that human and pig ascarids fall within the same gene cluster as all pig-derived ascarids, and they conjectured the infection in children had been acquired possibly through exposure to pig manure, thus implying the existence of cross-infection between pigs and humans. In a related study, despite the existence of ascarid gene-clustering showing striking differences between populations, the clusters were believed to have been highly influenced by current hybridisation between human and pig ascarids (CRISCIONE et al., 2007).

The similar protein banding patterns between human and hog ascarids detected in the present study is supportive of earlier studies that strengthen the supposition of the existence of cross-infection and the likelihood of natural hybridisation between the species (CRISCIONE et al., 2007; NEJSUM et al., 2005; ZHU et al., 1999). It is thus essential for control strategies to take into consideration the simultaneous treatment of infected individuals and the deworming of hogs. To assess further the probability of cross-

infection between *A. lumbricoides* and *A. suum*, additional epidemiological surveillance of ascariasis is highly recommended in rural and suburban places, where backyard hog-rearing is still commonly practiced to this day. Additional molecular studies should be able to aid in the elucidation of the species hybridisation potential.

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SAŽETAK

Ascaris lumbricoides i *Ascaris suum* bili su analizirani radi utvrđivanja sličnosti i razlike u elektroforetskoj pokretljivosti proteina iz njihove kutikule i spolnih organa. U obje vrste dokazana je znatna sličnost proteinskoga profila. Elektroforezom iscrpka kutikule ženki ustanovljeno je deset zajedničkih vrpci za obje vrste, dok je elektroforezom proteina kutikule mužjaka dobiveno 12 zajedničkih vrpci proteina od čega je najmanje pet proteina bilo zajedničko za oba spola. Usporedbom proteinskoga profila spolnih organa ženki i mužjaka obiju vrsta dokazane su također mnoge sličnosti. U proteinskomu profilu vrste *Ascaris lumbricoides* dokazano je 11 proteinskih traka dok je u proteinskomu profilu vrste *Ascaris suum* dokazano 12 vrpci, od čega je šest bilo zajedničkih. Elektroforezom proteinskih iscrpaka spolnih organa i kutikule mužjaka dokazano je osam zajedničkih proteinskih vrpci, dok su istom usporedbom proteinskoga profila ženki dokazane samo dvije zajedničke proteinske vrpce. Nalaz proteinskih vrpci od 25 kDa, 20-21 kDa i 18 kDa gotovo u svim pretraženim proteinskim ekstraktima govori o njihovoj velikoj važnosti te mogućoj upotrebi u svojstvu markera. Dokazane

sličnosti u sastavu proteinskih ekstrakata u askarida izdvojenih iz ljudi i svinja govori o njihovoj bliskoj genetskoj srodnosti. S obzirom na ove nalaze i ranija istraživanja koja upućuju na zoonotsku prirodu askaridoze i veliku mogućnost križnih invazija ljudi i svinja, treba donijeti kontrolne mjere za istodobno dehelmintizaciju invadiranih ljudi i svinja. Vjerojatnost križne invazije ljudi i svinja vrstama roda *Ascaris* treba procijeniti na osnovi pretraga na askaridozu u mjestima gdje se svinje još uvijek drže po dvorištima. Ovo je prva analiza proteinskoga profila vrsta roda *Ascaris* na Filipinima.

Ključne riječi: proteinski profil, *A. lumbricoides*, *A. suum*, Filipini, elektroforeza u gelu