

## Research Article

# The Hamster Model for Identification of Specific Antigens of *Taenia solium* Tapeworms

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Humans acquire taeniasis by ingesting pork meat infected with *Taenia solium* cysticerci, which are the only definitive hosts of the adult stage (tapeworm) and responsible for transmitting the human and porcine cysticercosis. Hence, detection of human tapeworm carriers is a key element in the development of viable strategies to control the disease. This paper presents the identification of specific antigens using sera from hamsters infected with *T. solium* tapeworms analyzed by western blot assay with crude extracts (CEs) and excretion-secretion antigens (E/S Ag) obtained from *T. solium* cysticerci and tapeworms and extracts from other helminthes as controls. The hamster sera infected with *T. solium* tapeworms recognized specific bands of 72, 48, 36, and 24 kDa, in percentages of 81, 81, 90, and 88%, respectively, using the *T. solium* tapeworms E/S Ag. The antigens recognized by these hamster sera could be candidates to improve diagnosis of human *T. solium* taeniasis.

## 1. Introduction

*Taenia solium* is a cestode that causes taeniasis when the adult worm (tapeworm) is lodged in the human intestine and cysticercosis when the larval stage (cysticercus) establishes in the central nervous system, skeletal muscle, and other organs of both pigs and humans [1]. Taeniasis causes only mild symptoms in the human carrier, which accounts for the disease remaining frequently undiagnosed, whereas cysticercosis of the central nervous system (neurocysticercosis, NCC) causes clinical manifestations that range from mild symptoms to death [2–4].

A study performed in hospitalized patients at the National Institute of Neurology and Neurosurgery in Mexico City during the period from 1995 to 2009 revealed that the annual frequency for hospitalizations due to NCC remained constant, with approximately 2.4% of neurology consultations attributable to NCC, whereas mortality from this disease has diminished, which can be explained by an opportune diagnosis [5]. Seroprevalence studies show that up to 12% of the populations in endemic areas carry anticysticercus antibodies [6].

The mean prevalence of *T. solium* taeniasis in endemic countries is 4%, though in some countries a prevalence of up to 7% has been reported. Some studies suggest that the disease is more common among women and individuals between 30 and 40 years of age [7, 8]. Epidemiological studies in Central and Latin America show that in the last thirty years, Colombia, Honduras, and Guatemala are the countries with the highest annual frequency of taeniasis, whereas Costa Rica has the lowest number of cases [9]. On the other hand, in four communities in Guatemala (Quesada, El Tule, El Jocote, and Santa Gertrudis) in the period from 1991 to 1994, 92 positive cases were detected among 3,399 studied samples (2.7%), and 98% of the recovered parasites were *T. solium*. A 1% frequency was detected in children aged 6 months to 4 years [8, 10].

Identification of the *Taenia* species (*T. saginata*, *T. asiatica*, and *T. solium*) is fundamental in the control and prevention of taeniasis/cysticercosis transmission in endemic areas. The methods used for these studies have been based on microscopic detection of eggs in feces [11] and on the detection of tapeworm coproantigens [12]. Diagnosis of

tapeworms through coproparasitoscopic analysis lacks sensitivity and specificity since the eggs of different taenias are morphologically indistinguishable, and although the detection of coproantigens is specific for the genus, it does not distinguish between species. However, at the experimental level, techniques based on DNA identification are capable of distinguishing between species of taenias [13].

Taeniasis in dogs has been diagnosed using ELISA test to detect antibodies that recognize components in the tapeworms excretion/secretion antigens (E/S Ag) [14, 15]. Recently, two serological methods (ELISA and IET) have been used for detection of *T. solium* taeniasis. These methods use two antigens obtained from adult stage (tapeworms) E/S Ag of *T. solium*: ES33 and ES38 and their recombinants (rES33 and rES38) which give sensitivity and specificity above 97% and 91%, respectively [16, 17].

Epidemiological studies show that human with *T. solium* taeniasis is the risk factor in the transmission of cysticercosis in pigs and humans [6, 18]. However, little effort has been made to identify new specific antigens in order to develop a low-cost, feasible method, which is both sensitive and specific for detecting tapeworm carriers. Thus, the present study was carried out in order to identify specific antigens from *T. solium* tapeworm, using the *T. solium* taeniasis-hamster model.

## 2. Materials and Methods

**2.1. Biological Material.** *Taenia solium* cysticerci and tapeworms were obtained by dissecting them from skeletal muscles from naturally infected pigs and from small intestine of experimental infected hamster. Animals were processed according to the Official Mexican Norms: NOM-009-ZOO-1994 for sanitary processing of meats and NOM-033-ZOO-1995 for humanitarian sacrifice of domestic and wild animals. Cysticerci were washed in cold sterile phosphate-buffered saline (PBS), pH 7.4. Viability of cysticerci in each lot was determined by incubation of 20 cysticerci at 37°C in RPMI 1640 medium (Sigma) complemented with pig bile at 25% for 24 h [19]. Cysticerci were considered viable, when the scolex of the larva evaginates and displays contractile movements. The number of evaginated parasites was counted and a mean percentage of viability was established.

**2.2. Development *Taenia solium* Tapeworms.** Golden hamsters (*Mesocricetus auratus*) were treated orally with praziquantel (PQZ 3 mg/animal). A week later, the animals were infected orally with eight cysticerci [20]. Briefly, animals were immunosuppressed by intraperitoneal administration of methylprednisolone (2 mg/animal) every 15 days. Five weeks later, the animals were killed by intraperitoneal administration of sodium pentobarbital (200 mg/kg). *Taenia solium* tapeworms were recovered from the small intestine, washed with antibiotic complemented PBS (penicillin  $1 \times 10^6$  U/L and streptomycin 2 g/L, Sigma), and cultivated in RPMI medium to obtain the E/S Ag. Blood samples were obtained from the hamsters before infection to be used as a negative control serum (preimmune serum) and

shortly before killing. Blood samples were incubated at room temperature for 30 min and centrifuged at 735 g for 10 min to obtain serum. The recovered sera were stored at  $-20^\circ\text{C}$  until needed.

### 2.3. Antigens Preparation

**Cysticerci E/S Ag.** Cysticerci were incubated for 6 hours at room temperature in Petri dishes containing RPMI medium with antibiotic and EDTA (1 mM). The culture medium was recovered and centrifuged at 9000 g for 20 min, the supernatant was filtered through 0.45  $\mu\text{m}$  membranes (Millipore), dialyzed against PBS, and concentrated in an AMICON unit using 3000 PM membranes (Millipore). Protease inhibitors (TLCK, PMSF, and EDTA) were added and the antigens were stored at  $-20^\circ\text{C}$  [21].

**Tapeworm E/S Ag.** Tapeworms were incubated at 37°C in Petri dishes containing RPMI medium with antibiotic (penicillin  $1 \times 10^6$  U/L and streptomycin 2 g/L, Sigma). The medium was exchanged every 8 hours on the first day of incubation and every 12 hours during the following 3 to 4 days. Viability of tapeworm was monitored daily through microscopic observations of morphology and mobility. The media recovered from the incubation were pooled and centrifuged at 9000 g for 20 min. The supernatant was dialyzed against PBS, concentrated, protease inhibitors (TLCK, PMSF, and EDTA) added and the samples were frozen at  $-20^\circ\text{C}$  until used [17].

**Crude Extracts (CE).** The parasites (*Taenia solium*, *Taenia saginata*, *Taenia taeniaeformis*, *Hymenolepis diminuta*, and *Ascaris lumbricoides*) were homogenized individually by a polytron (Brinkmann Instruments, Inc) at maximal power for 3 min in PBS, pH 7.4, using a ratio of 1 g parasite per 5 ml of extraction solution, complemented with protease inhibitors. The suspension was centrifuged at 19,870 g for 30 min and the supernatant was dialyzed against PBS at 4°C, overnight. The resulting mixture was ultracentrifuged at 100,000 g for 30 min; the supernatant was distributed in aliquots and frozen at  $-20^\circ\text{C}$ .

**Recombinant Antigens.** We isolated clones that code for the P29 and antigen B (AgB or paramyosin) antigens by screening an expression library of *T. solium* adult stage constructed in  $\lambda$ ZAPII vector with a hyperimmune rabbit serum anti-CE of *T. solium* tapeworm. Recombinant AgB preparation was carried out according to a previously established protocol [22], and the *T. solium* P29 antigen (TsP29) was produced using the pRSETB vector and the recombinant antigen purified by metal affinity chromatography [Jiménez et al., unpublished results]. The concentration of purified proteins was determined by the Lowry method and diluted at 1 mg/ml in PBS [23].

**Production of Hyperimmune Sera.** Hyperimmune sera against *Taenia solium* (cysticerci and tapeworm crude extracts, E/S Ag, AgB, and P29 antigens) were prepared in eight-week-old female hamsters. Prior to immunization,

TABLE 1: Percentages and recovery of *Taenia solium* tapeworm in immunosuppressed hamsters.

Cysticerci lot	Hamsters used/infected	Infection (%)	Tapeworms recovered (size)	Recovery (%)
1	13/9	69.23	37 (10–30 cm)	36
2	7/3	42.85	10 (20–30 cm)	18
3	10/9	90	37 (10–30 cm)	46
4	18/15	83.33	52 (15–40 cm)	36
Total	<b>48/36</b>	<b>74.35</b>	<b>134</b>	<b>34</b>

blood samples were taken to obtain a preimmune serum to serve as negative control in western blot assays. Hamsters were immunized subcutaneously with 50  $\mu$ g of each antigen/animal, every 15 days. After the final immunization, sera were obtained, antibody titers were determined by ELISA and stored at  $-20^{\circ}\text{C}$ . Hyperimmune sera against CE of known helminthes were obtained from rabbits and prepared according to the same immunization scheme.

**Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).** The electrophoretic patterns of the CE and E/S Ag of *T. solium* cysticerci and tapeworms were determined employing 50 and 15  $\mu$ g, respectively, in a 10% SDS-PAGE adding 2-mercaptoethanol as a reducing agent [24]. Electrophoresis was carried out in a MiniProtean II (Bio-Rad) camera at 100 V. The crude extract gels were stained with Coomassie brilliant blue and the E/S Ag gels were silver-stained.

**Western Blot Assay.** We carried out preparative 10% SDS-PAGE using 2  $\mu$ g of protein for the crude extracts or E/S Ag and 200 ng of pure antigens for every linear millimeter of gel as well as a prestained molecular weight marker (Benchmark Prestained protein ladder, Invitrogen). The antigens were transferred onto nitrocellulose membranes (Hybond-C, Amersham Biosciences) using a Bio-Rad Mini Transblot equipment (100 V/1 h) in cold. The membrane was washed with PBS, cut into 2 mm wide strips, and stored at  $-20^{\circ}\text{C}$ .

For the identification of specific tapeworm antigens, the membranes with the different extracts were confronted with the sera from hamsters infected with *T. solium* tapeworms and preimmune sera (1:100), as well as with the hyperimmune hamster and rabbit sera (1:1000) diluted in PBS-Tween 0.3% and 5% fat-free milk. The membranes were incubated for 1 h at room temperature under constant agitation and subsequently washed three times for 5 min with PBS-Tween 0.3%. A second peroxidase-conjugated hamster or rabbit anti-IgG antibody (Zymed) at a 1:2000 dilution was added and incubated for 1 h under constant stirring at room temperature. The membrane was washed as previously, and the antibodies bound to the membranes were developed using diaminobenzidine (5  $\mu$ g/ml) and 0.3%  $\text{H}_2\text{O}_2$ . To discard cross-reactions to other helminthes, membranes containing extracts from *T. saginata*, *T. taeniaeformis*, *H. diminuta*, and *A. lumbricoides* were tested against the same sera. Bands obtained in membranes by western blot were analyzed with the 1D Image Analysis Software (Kodak Digital Science).

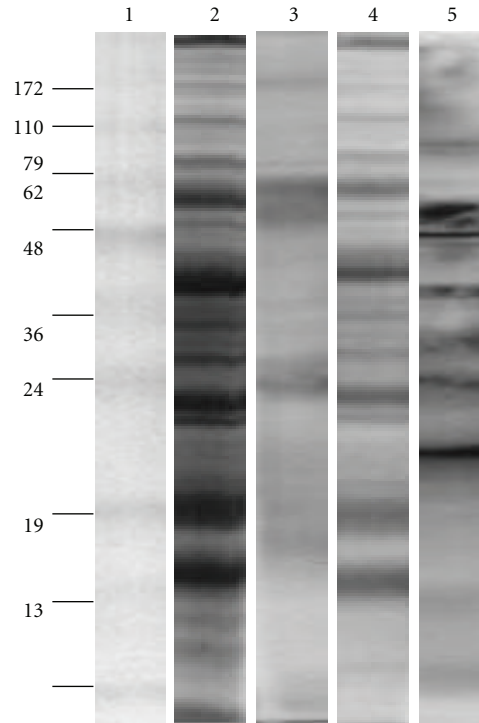


FIGURE 1: 10% SDS-PAGE. Lane 1: molecular weights (Benchmark prestained protein ladder, Invitrogen), and *Taenia solium*. Lanes 2: tapeworm crude extract (CE), Lane 3: tapeworm excretion/secretion antigens (E/S Ag), Lane 4: cysticerci CE, Lane 5: cysticerci E/S Ag. Lanes 2 and 4 stained with Coomassie blue, lanes 3 and 5 with silver stain.

### 3. Results

We obtained several lots of cysticerci from naturally infected pigs acquired from different regions in Mexico. Lots presented 90, 85, 66, and 90% of scolex evagination and a mean viability percentage of 83%. Table 1 shows that, at the time of death (5 weeks), the number and length of recovered tapeworms vary in each lot, with a mean number of three tapeworms recovered from each hamster and a mean length of 25 cm. Recovered tapeworms and cysticerci were incubated in RPMI medium supplemented with antibiotics to obtain the E/S Ag.

The composition of CE from *T. solium* cysticerci and tapeworms observed in 10% SDS-PAGE stained with Coomassie blue shows very similar complex patterns (Figure 1, lanes 2 and 4), with bands between 13 to 200 kDa.

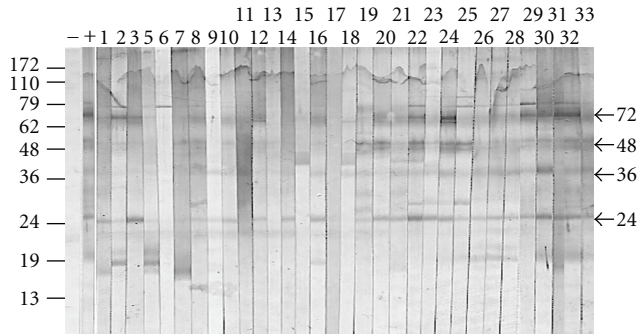


FIGURE 2: Western blot with tapeworm E/S Ag of *Taenia solium* was incubated with hamster sera infected with *T. solium* tapeworms (1–33). Preimmune (lane –) and hyperimmune hamster sera antitapeworm E/S Ag of *T. solium* (lane +) were used as negative and positive controls. Peroxidase-conjugated antihamster IgG antibody was used as second antibody.

TABLE 2: Recognition percentage of the different bands in tapeworm E/S Ag of *Taenia solium* by 33 hamster sera infected with *T. solium* tapeworms.

Band (kDa)	Serum number	Recognition (%)
85	4 (23,24,25,26)	9
80	C+, 15 (2,6,8,19,22,24–33)	45
77	C+, 23 (2,5,6,8,10,12,16,20,23–26,27,29–33)	69
72	C+, 27 (2–5,7,8,10–12,15,16,18–33)	81
52	C+, 16 (2–8,19–25,29,32)	48
48	C+, 27 (2–5,7,8,10–14,16,19–33)	81
43	4 (15,18,21,22)	12
36	C+, 30 (1–5,7–14,16,18–33)	90
30	C+, 5 (19,21,24, 32,33)	15
27	C+, 4 (22,23,24,25)	12
25	C+, 1 (19)	3
24	C+, 29 (1–5,7–12,14,16,17,19–33)	88
22	C+, 22 (1–14,16–20,26,27,31)	66
19	C+, 4 (2,5,27,28)	12
17	C+, 10 (2,5,16,25–28,30,32,33)	30
15	4 (1,2,7,29)	12

In contrast, the silver-stained patterns from *T. solium* tapeworms (lane 3) and cysticerci (lane 5) E/S Ag are different among themselves and different from patterns obtained for the CE of the tapeworms (lane 2) and cysticerci (lane 4). In the case of the tapeworm E/S Ag, eight bands ranging from 20 to 170 kDa were observed, the 62–78, 48, 36, and 20–24 kDa bands being distinct. For the cysticerci's E/S Ag the recognizing were in the region of 30–110 kDa, with five distinct bands of 64, 50, 40, 30, and 28 kDa and a doublet in the 90 kDa region (lane 5).

When the 33 hamster sera infected with *T. solium* tapeworms (lanes 1–33) were tested against tapeworm *T. solium* E/S Ag, sixteen bands of different molecular weight

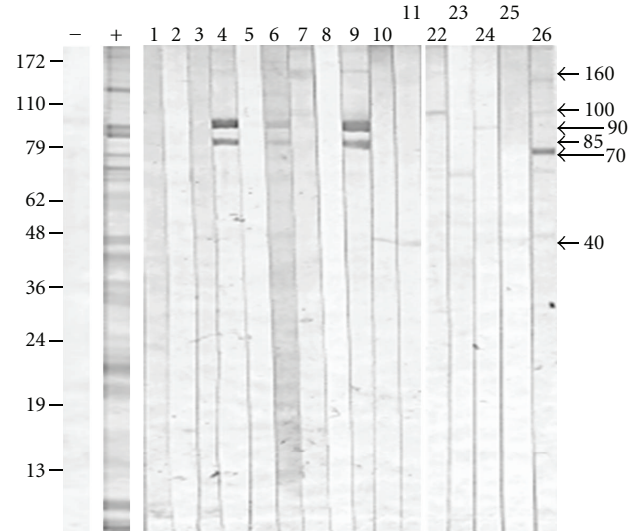


FIGURE 3: Western blot with *Taenia solium* tapeworm CE was tested with hamster sera infected with *T. solium* tapeworms (1–33). Preimmune (lane –) and hyperimmune hamster sera against *T. solium* tapeworm CE (lane +) were used as negative and positive controls. WB shows representative samples of the assay. Number of lanes is corresponding to serum number of the assay.

TABLE 3: Recognition percentages of the different bands in CE of the *T. solium* tapeworms by 33 hamster sera infected with *T. solium* tapeworms.

Band (kDa)	Serum number	Recognition (%)
160	C+, 8, 10, 26	9
100	C+, 8, 16, 20, 22	12
90	C+, 5, 7, 10, 24	12
85	C+, 5, 7, 10	9
70	C+, 26	3
40	C+, 11, 12, 13, 15, 18, 19, 24, 25, 26	27

were easily distinguished between the 13 to 172 kDa. However, four bands in the regions of 72, 48, 36, and 24 kDa were recognized in 81, 81, 90, and 88%, respectively. It is worth mentioning that the preimmune sera used as negative controls (lane –) reacted mildly with a band in the 20 kDa region, and this band was discarded from further analysis. The positive control (lane +), a hyperimmune anti-tapeworm E/S Ag hamster serum, reacted intensely with bands between 20 and 172 kDa, as well as with the 72, 48, 36, 30, 24, and 20 kDa bands, recognized by the sera from hamster infected with *T. solium* tapeworms (Figure 2, Table 2).

The results of western blot with CE of *T. solium* tapeworm and the hamster sera infected with *T. solium* tapeworms show that hamster sera recognized bands in the range of 40 to 160 kDa, with 40 kDa band being one of the most recognized (27%). Four bands (160, 100, 90, and 70 kDa) were recognized by 3 to 12% of the sera. The negative control (lane –), preimmune serum, did not show any reaction, whereas the positive control (lane +), a hyperimmune



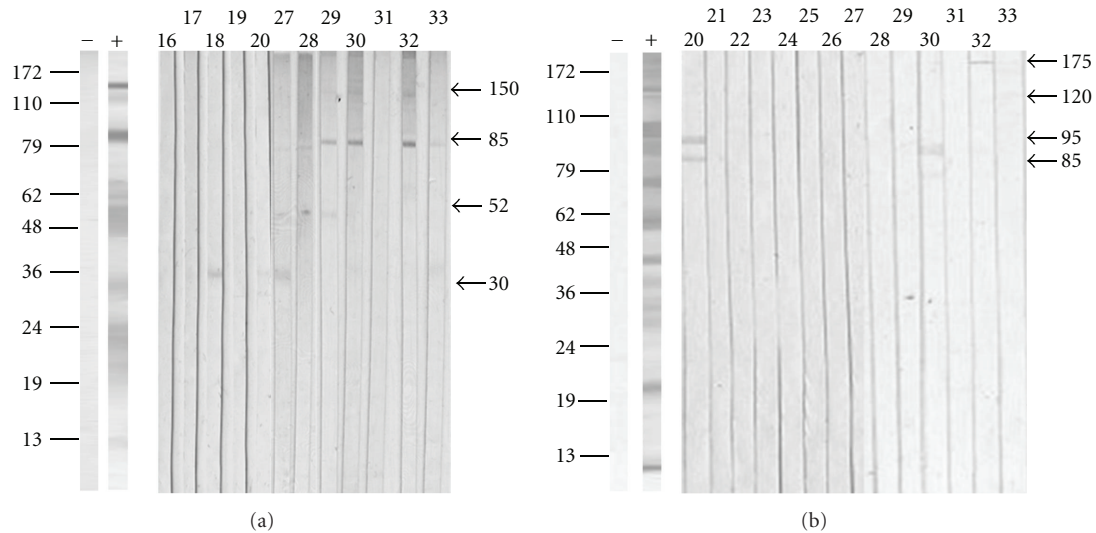


FIGURE 4: (a) Western blot with *Taenia solium* cysticerci E/S Ag was tested with 33 hamster sera infected with *T. solium* tapeworms (1–33). Preimmune (lane –) and hyperimmune hamster sera anti-*T. solium* cysticercus E/S Ag (lane +) was used as negative and positive controls. WB shows representative samples of the assay. Number of lanes is corresponding to serum number of the assay. (b) Western blot with *Taenia solium* cysticercus CE was tested with 33 hamster sera infected with *T. solium* tapeworms (1–33). Preimmune (lane –) and hyperimmune hamster sera anticysticercus CE (lane +) were used as negative and positive controls. WB shows representative samples of the assay. Number of lanes is corresponding to serum number of the assay.

hamster serum antitapeworm CE, recognized several bands in the range of 10 to 170 kDa (Figure 3, Table 3).

On the other hand, in the hamster sera infected with *T. solium* tapeworms in the E/S Ag of *T. solium* cysticerci, only four bands of 150, 85, 52, and 30 kDa were recognized in 6, 21, 9, and 27%, respectively. It should be noted that these bands and others in the 19 to 200 kDa range were also recognized by the hyperimmune hamster serum anticysticercus E/S Ag used as a positive control (lane +). Conversely, the preimmune serum did not recognize any bands on the membrane (lane –) (Figure 4(a), Table 4(a)).

When cysticerci CE antigens were used in membrane, only three hamster sera infected with *T. solium* tapeworms reacted with the 175, 120, 95, and 85 kDa bands, which represent recognition of 3, 3, 6, and 6%, respectively. Preimmune (lane –) hamster serum did not react with any component of the CE cysticerci. The positive control (lane +) recognized membrane bands in the range of 10 to 200 kDa, showing a strong response with the 95, 85, 70, 60, 45, 30, 20, and 13 kDa bands (Figure 4(b), Table 4(b)).

Since hamster sera infected with *T. solium* tapeworms frequently recognized bands in the 85, 90, and 29 kDa regions and because AgB and P29 antigens are located in these regions, and have been used for diagnosis of cysticercosis and echinococcosis [25, 26], we performed a western blot using the 33 hamster sera infected with *T. solium* tapeworms with these proteins of *T. solium*. Ninety-six percent of the infected hamster's sera recognized 85 and 95 kDa bands at different intensities and showed slight reaction to some bands below this range (Figure 5(a)), while the same sera showed a 28% recognition of the 29 kDa band (Figure 5(b)). The hyperimmune anti-AgB and anti-P29 sera, used as positive controls, specifically recognize the 85, and 95 (antigen B)

TABLE 4: (a) Recognition percentage of *T. solium* cysticerci E/S Ag by 33 hamster sera infected with *T. solium* tapeworms. (b) Recognition percentage of the 33 hamster sera infected with *T. solium* tapeworms on antigens of *T. solium* cysticerci CE.

(a)		
Band (kDa)	Serum number	Recognition (%)
150	C+, 30,32	6
85	C+, 5, 27–30,32,33	21
52	C+, 27,28,29	9
30	C+, 1,3,12,14,18,20,26,30,33	27
(b)		
Band (kDa)	Serum Number	Recognition (%)
175	C+, 32	3
120	C+, 33	3
95	C+, 20,30	6
85	C+, 20,30	6

and the 29 kDa (P29 antigen) bands, as well as other lower weight bands presumably representing degradation products of these antigens.

The results of the western blot assay with CE of other helminthes show that two hamster sera infected with *T. solium* tapeworms recognized a band of approximately 100 kDa in the CE of *T. saginata* (Figure 6(a)). In the case of CE from *T. taeniaeformis*, we observed that three sera reacted with a band of approximately 100 kDa showing 9% recognition (Figure 6(b)). In the case of *H. diminuta*,

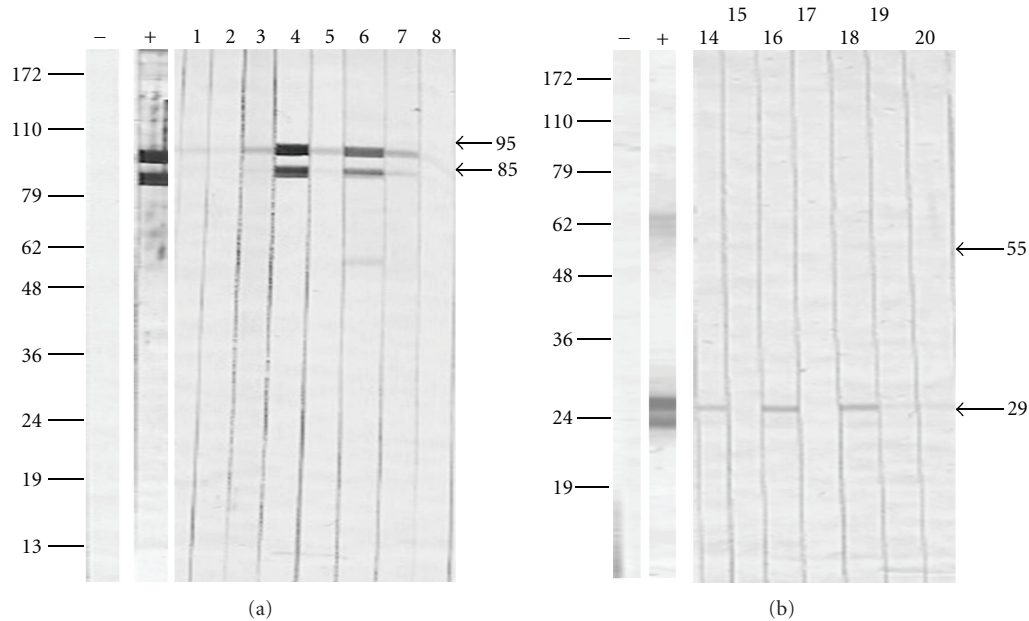


FIGURE 5: Western blot with AgB (a) and P29 (b) recombinants from *Taenia solium* was incubated with 33 hamster sera infected with *T. solium* tapeworms. Preimmune hamster normal sera (lane -) were used as negative controls. Hyperimmune anti-AgB and anti-P29 from *T. solium* sera from hamster were used as positive controls (lanes +). WB shows representative samples of the assay. Number of lanes is corresponding to serum number of the assay.

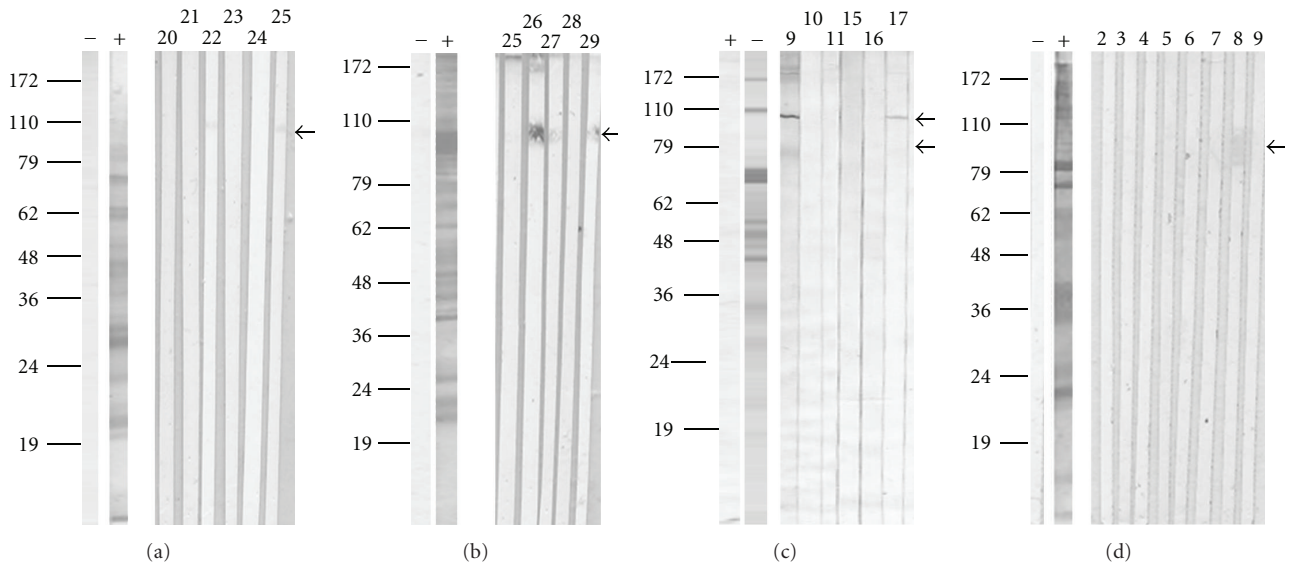


FIGURE 6: Western blot shows cross-reaction with other helminthes. Crude extracts (CE) from (a) *Taenia saginata*, (b) *Taenia taeniaeformis*, (c) *Hymenolepis diminuta*, and (d) *Ascaris lumbricoides* were confronted with 33 hamster sera infected with *T. solium* tapeworms. Hyperimmune sera prepared in rabbits against each of the CE were used as positive controls (lanes +) and preimmune sera from rabbit were used as negative controls (lanes -). WB shows representative samples of the assay. Number of lanes is corresponding to serum number of the assay.

the sera reacted primarily with bands in the 100, 81, and 50 kDa regions (Figure 6(c)). Additionally, only one serum recognized bands in the 80 and 100 kDa region in the CE of *Ascaris lumbricoides* (Figure 6(d)). The preimmune hamster (data not shown) and rabbit sera used as negative controls (lane -) showed no reaction in the membranes with CE from these helminthes; in contrast, hyperimmune rabbit sera (lane +) against CE of helminthes recognized bands in the 20

and 172 kDa range, when tested against CE of *T. saginata*, *T. taeniaeformis*, *H. diminuta*, and *A. lumbricoides*.

#### 4. Discussion

In view of the continuing threat to public health represented by NCC, numerous studies have been conducted to develop better and cheaper diagnostic methods [27]. Currently,

there are different serological methods available that employ ELISA and western blot using *T. solium* cysticercus glycoprotein isolated with *Lens culinaris* or its recombinants. These methods have 98% sensitivity and 100% specificity in serum [28, 29]. In contrast, diagnosis of taeniasis has not received the same attention. There are several reasons for this: taeniasis remains frequently unrecognized by the host since the symptoms are usually mild and unspecific, and the high risk involved handling the tapeworm eggs [1, 30]. To eliminate the risk of exposure to human feces contaminated with *T. solium* eggs, we used the taeniasis model in golden hamsters. This model is safe since the tapeworms that develop in the hamster's intestine do not reach sexual maturity and do not produce infective eggs [20, 31]. Table 1 shows that in immunosuppressed animals infected with eight cysticerci, the rate of established parasites is 34.89%, with variable permanence at 5 weeks of infection, a rate which is in accord with previous publications [31, 32].

The 10% SDS-PAGE assays show that all fractions present great complexity. For the CE, we observed a similar pattern for both the cysticercus and tapeworms showing bands in the range of 13 to 200 kDa. Conversely, we observed great differences in the banding patterns of E/S Ag between the cysticercus and the tapeworm. In the E/S Ag from the tapeworm, 12 bands in the 13 to 110 kDa were distinguished, and these results are similar to those reported by Wilkins and coworkers [17], who identified approximately 16 bands. For the cysticercus E/S Ag, we found 20 bands in the 13 to 172 kDa range, with distinct bands of 64, 54, 46, and 30 kDa. These data are also consistent with previous findings of 13 to 19 bands described in the cysticercus E/S Ag [33, 34].

The western blot assays on membranes containing *T. solium* tapeworm E/S Ag confronted by the 33 hamster sera infected with *T. solium* tapeworms identified four antigens of 72, 48, 36, and 24 kDa with a recognition of 81, 81, 90, and 88%, respectively. Wilkins and coworkers [17] showed that sera from *T. solium* tapeworm-infected patients confronted by adult stage E/S Ag detected two bands of 32.7 and 37.8 kDa. The 37.8 kDa band matches with the 36 kDa band identified in this paper by hamster sera infected with *T. solium* tapeworms and presumably corresponds to the same antigen.

To determine the specificity of the identified bands, we performed a western blot assay by confronting the hamster sera infected with *T. solium* tapeworms with cysticerci E/S Ag and with the CE from both cysticerci and tapeworms of *T. solium*, which showed that four bands were recognized, 150, 85, 52, and 30 kDa, with the 30 kDa bands showing the highest percentage of recognition (27%). This band does not match with the bands identified by the hamster sera infected with *T. solium* tapeworms in the tapeworm E/S Ag. Additionally, the hamster sera infected with *T. solium* tapeworms frequently recognized two other bands at varying intensities and corresponding to a 95–85 kDa band in the case of cysticerci CE and a 29 kDa band in the case of E/S Ag of cysticerci and tapeworm. These bands match with two well-known diagnostic antigens of cysticercosis and echinococcosis, AgB and P29 [25, 26]. However, these antigens are not

recommended for the specific diagnosis of *T. solium*, since they are both present in other helminthes [26, 35].

In this paper, we observed that hamster sera infected with *T. solium* tapeworms reacted mildly with 100 kDa in the CE from *T. saginata*, *T. taeniaeformis*, *H. diminuta*, and *A. lumbricoides*. However, there are reports of the lack of cross-reactions using *T. solium* tapeworms E/S Ag and human sera with other parasitic infections, including *T. saginata* [17, 27].

It is worth mentioning that tapeworm-infected hamsters, whether immunosuppressed or not, produce antibodies against *T. solium* from the first week of infection and titers increasing as the infection is prolonged to finally decrease after the worm is expelled [32, 36]. This suggests that these bands (72, 42, 36, and 24 kDa) could be used to detect serum antibodies to human tapeworms as well as to produce antibodies that detect them in samples of patients with taeniasis. In addition, the recombinant forms of these bands could improve the suggested method for diagnosis of taeniasis [17].

Humans are the only definitive host of *T. solium* tapeworm and, thus, responsible for causing both human and swine cysticercosis [6, 18]. It is also known that humans can be easily treated for this parasitosis by antihelminthic drugs, such as praziquantel and albendazole [12, 37, 38]. Therefore, further studies should be undertaken with sera from human patients with taeniasis or in endemic areas to determine if these antigens could be candidates for the specific detection of *T. solium* taeniasis. In developing countries, where taeniasis-cysticercosis is an endemic disease and continues to be a significant public health problem, it is essential to have reliable and cheap diagnostic tools to screen populations, as a primary measure for control and eradication programs [5, 38].

## 5. Conclusions

We identified four specific antigens in the *T. solium* tapeworm E/S Ag that are useful in the detection of tapeworms in hamsters, which could be potentially useful in the diagnosis of human *T. solium* taeniasis.

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