Parasitol Res (2013) 112:2069–2073
DOI 10.1007/s00436-013-3368-5

ORIGINAL PAPER

An immunoblot for detection of *Taenia saginata* cysticercosis

Sameh Abuseir · Uschi Nagel-Kohl · Sonja Wolken · Christina Strube

Received: 31 January 2013 / Accepted: 21 February 2013 / Published online: 13 March 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Control measures to prevent human infections with the food-borne zoonotic helminth *Taenia saginata* are currently based on meat inspection, which shows rather low diagnostic sensitivity. To develop an immunoblot for detection of *T. saginata*-infected cattle, crude proteins of *T. saginata* cysts were extracted and separated with SDS-PAGE. The cyst antigens showed ten protein bands ranging from 260 to 14 kDa. *T. saginata* cyst proteins 260, 150, 130, 67, 60, 55, 50, and 23 kDa were immunoreactive with known positive sera of *T. saginata*-infected cattle but cross-reacted with sera from *Echinocccus granulosus*-infected ruminants. By contrast, 14- and 18-kDa cyst proteins reacted specifically with *T. saginata*-positive sera and thus might be potential candidates for the development of a *T. saginata*-specific immunoassay. Proteins of *E. granulosus* cysts and *Taenia hydatigena* cysts were also extracted and separated with SDS-PAGE. *E. granulosus* cysts revealed 11 protein bands ranging from 260 to 23 kDa. *E. granulosus* protein 60 kDa was immunoreactive with *E. granulosus*-positive sera only. The cyst of *T. hydatigena* showed 11 protein bands ranging from 290 to 14 kDa. The protein band 35 kDa showed cross-reaction with positive sera from both *T. saginata*- and *E. granulosus*-infected animals. A protein of 67 kDa was present in all three tested cestode species and was the major antigenic protein detected by sera of *T. saginata*- and *E. granulosus*-infected animals. Therefore, this protein represents a potential vaccine candidate against both cysticercosis and cystic echinococcosis in cattle.

S. Abuseir (✉)
Faculty of Veterinary Medicine, An-Najah National University, P.O. Box 7, Nablus, West Bank, Palestine
e-mail: sameh.abuseir@gmail.com

U. Nagel-Kohl
Lower Saxony State Office for Consumer Protection and Food Safety, Food and Veterinary Institute, Hannover, Germany

S. Wolken · C. Strube
Institute for Parasitology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, Hannover, Germany

Introduction

Taenia saginata cysticercosis represents the parasitic larval stage of the beef tapeworm belonging to the Taeniidae family of tapeworms (Cestoda). Humans are the final and bovines the intermediate host to this infection. Human taeniosis, or infection with the adult *T. saginata* worm, is characterized by the presence of the between 3.5- and 20-m-long worm in the small intestine of the infected person, who may pass millions of eggs daily. These eggs may survive for months in an appropriate environment. Estimates based on computations from incidence indicate that 2 % of the human population in Europe is infested with *T. saginata* (SCVPH 2003).

Bovine cysticercosis is the infestation of the intermediate host "cattle," which suffer from cysticerci/cysts of *T. saginata* in their muscles and tissues. As it has been reviewed by the European Commission (SCVPH 2000), the prevalence of bovine cysticercosis in various European countries based on slaughterhouse data varies between 0.01 and 6.8 %. The real prevalence is expected to be three to ten times higher than the estimated prevalence (Abuseir et al. 2010; Dorny et al. 2000; Onyango-Abuje et al. 1996; Van Knapen and Buys 1985; Geerts et al. 1981). Light infestations of the carcasses are unlikely to be detected during meat inspection, because cysts are frequently found in other locations than the predilection sites usually examined (Eichenberger et al. 2011; EFSA 2006; Minozzo et al. 2002; SCVPH 2000). Overall, there is a need for development of more sensitive methods than meat inspection to detect *T. saginata* cysticercosis in beef.

Cystic echinococcosis caused by *Echinococcus granulosus* is one of the most important zoonotic cestode infections causing significant morbidity and mortality in humans as well as significant economic losses in livestock animals (Casulli et

al. 2008, EFSA 2011). Cross-reactivity between *Taenia* and *Echinococcus* cysts has been demonstrated (Flávia de Assunção et al. 2010) as they share antigenic components (Ishida et al. 2003). Therefore, it is necessary to carry out procedures to select immunogenic components specific for *T. saginata*. In the present study, the antigenic pattern of cyst material from *T. saginata*, *E. granulosus*, and *Taenia hydatigena* was examined by immunoblot analysis to identify specific antigens with diagnostic potential for *T. saginata* cysticercosis.

Materials and methods

Sample collection

T. saginata cysts (n=50) were collected from cattle slaughtered at an abattoir in northern Germany. The cysts were collected during routine meat inspection procedures. Each sample was kept in a special container, refrigerated at 4 °C, and transferred within 24 h to the laboratories of the Veterinary Institute, Hannover, Lower Saxony State Office for Consumer Protection and Food Safety, for further examination. The cysts were examined macroscopically and classified accordingly as viable or degenerated (Abuseir et al. 2006; Minozzo et al. 2002). Crude antigen preparation and protein extraction were performed from mature *T. saginata* cysts only.

T. saginata-positive serum samples were collected from cattle (n=10) experimentally infected with 5,000–10,000 *T. saginata* eggs by the Agricultural Research Institute, Muguga, Kenya. Animal experiments were approved by the Kenya Agricultural Research Institute Animal Care and Use Committee and were performed in compliance with the guidelines published by Kenya Veterinary Association and Kenya Laboratory Animal Technician Association. Serum samples were tested for positivity with an evaluated ELISA (Abuseir et al. 2006).

E. granulosus cysts (n=10) and *T. hydatigena* cysts (n=10) were collected from sheep and cattle slaughtered in an abattoir at the northern part of Palestine. Hydatid fluid was aspirated from the *E. granulosus* cysts and examined for the presence of protoscolices. Sterile cysts were excluded from subsequent protein extraction. *E. granulosus* cysts were sent to the laboratories of An-Najah National University, Palestine, for DNA isolation and subsequent species confirmation described by Adwan et al. (2013). In short, protoscolices or germinal membranes were used for DNA extraction. Amplification of DNA and confirmation were carried out by PCR targeting the cytochrome c oxidase subunit 1 (*cox1*) gene. Positive serum samples were collected from sheep (n=8) and cattle (n=2) naturally infected with *E. granulosus*.

Liver fluke-positive serum samples were collected from naturally infected cattle showing high antibody titers against *Fasciola hepatica* (n=10), which is one of the most common parasites found in cattle and thought to show cross-reactions with *T. saginata* metacestodes (Dennis et al. 1993; Baumeister et al. 1995).

Negative serum samples originated from the serum library at the Institute for Parasitology of the University of Veterinary Medicine Hannover. Sera were collected from parasite-naïve calves aged 3–6 months (n=100) that were reared under strict hygienic conditions for experimental use at the institute's animal facility.

Extraction of cyst proteins

The cysts were chopped with a scissor and then homogenized at 4 °C in 1/5 wt/vol PBS (pH 7.4) containing protease inhibitors (10 mM PMSF and 2.5 mM leupeptin). Homogenization was done in three steps: In the first step, the chopped cysts were disrupted in a tissue lyser (TissueLyser, Qiagen) with three stainless steel beads at a shaking speed of 30/s for 3 min. The samples were then further homogenized with a Bandelin Sonophus ultrasound HD 2070 at 40 % power for 1 min. Finally, the samples were stirred at 4 °C for 2 h and then centrifugated at 15,000×g for 1 h. The soluble extract was then collected, and protein concentration was estimated by the method of Bradford (1976).

SDS-PAGE and immunoblot

SDS-PAGE and immunoblot were done as described previously (Hyeong-Kyu and Keeseon 2009; Tsang et al. 1989) with modifications. SDS-PAGE was conducted in 6, 8, 10, 12, and 15 % gel in a Hoefer Mighty Small II SE 260 Mini-Vertical Gel Electrophoresis Unit (Amersham Pharmacia Biotech). Per lane, 30–40 μg of protein of the cyst samples were loaded. Prestained protein standards were from Fermentas Life Science (PageRuler™ Plus Prestained Protein Ladder) and Roth (Roti®-Mark 10-150 Plus). Duplicate gels were usually done. After electrophoresis, the crude antigens in one gel were transferred to a nitrocellulose membrane in a Hoefer TE 22 Mighty Small™ Transphor Tank Transfer Unit (Amersham Biosciences). The duplicate gel was stained with Coommassie Blue dye to visualize proteins.

Nitrocellulose membranes were incubated for 1 h in Roti®-Block working solution (Roth) after being washed for 5 min with Tris-buffered saline (TBS). The membranes were then washed three times for 5 min with TBS-Tween 0.05 % and incubated for 2 h at room temperature with sera diluted 1:100 in TBS-Tween 0.05 %. Antigen bands that reacted with serum antibodies were visualized with a monoclonal anti-bovine IgG secondary antibody conjugated to

alkaline phosphatase (Sigma) diluted 1:10,000 in TBS-Tween 0.05 %. Unbound antibodies were removed by three times washing with TBS. A premixed BCIP®/NBT substrate solution (Sigma) was used as substrate, and the enzyme reaction was stopped within 5 min with distilled water.

Results

SDS-PAGE and immunoblot of *T. saginata* cyst antigen

The SDS-PAGE profile followed by Coomassie Blue staining of *T. saginata* cyst antigens showed ten visible protein bands ranging from 260 to 14 kDa. These bands were approximately 260, 150, 130, 67 (a major band), 60, 55, 50, 23, 18, and 14 kDa. All protein bands of *T. saginata* reacted with *T. saginata*-positive sera (n=10) obtained from experimentally infected cattle. Testing the protein bands of *T. saginata* against positive *E. granulosus* sera (n=10) showed that only the 18- and 14-kDa proteins of *T. saginata* were not immunoreactive. Serum samples (n=10) from animals infected with *F. hepatica* showed no antigenic reactions with any extracted proteins of tested cestodes. All negative serum samples (n=100) showed no reaction with any of the extracted proteins. Results of SDS-PAGE and immunoblot are visualized in Fig. 1a.

SDS-PAGE and immunoblot of *E. granulosus* cyst antigen

The hydatid cysts were confirmed as *E. granulosus* by PCR targeting the *cox1* gene followed by sequencing of the amplification products (Adwan et al. 2013). The protein bands of *E. granulosus* resulting from cyst material ranged from 260 to

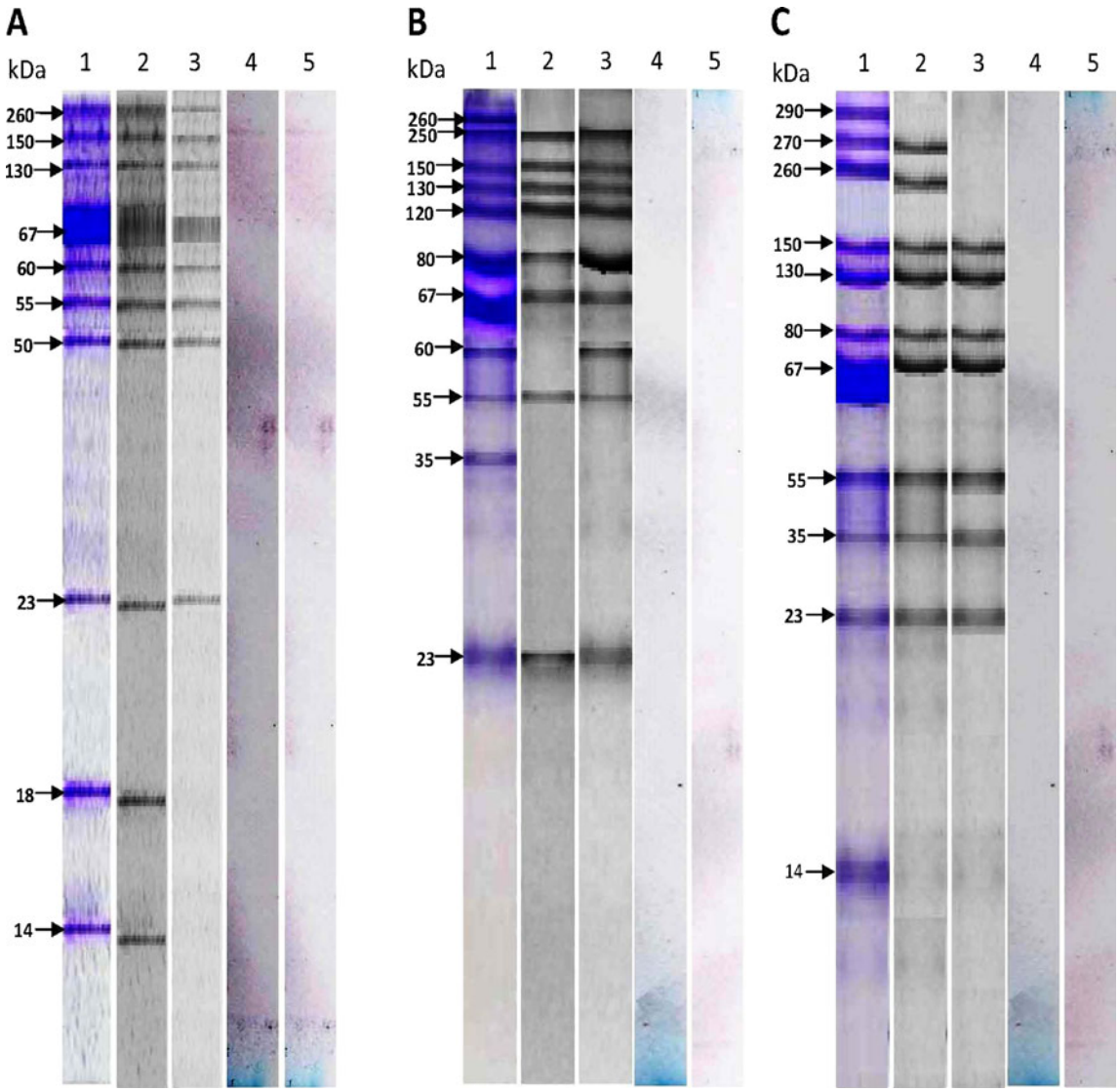

Fig. 1 SDS-PAGE (*lane 1*) of cyst proteins of *T. saginata* (**a**), *E. granulosus* (**b**), and T. hydatigena (**c**), and the immunoblot patterns against sera from ruminants infected with *T. saginata* (*lane 2*), *E. granulosus* (*lane 3*), and *F. hepatica* (*lane 4*) as well as sera from parasite-naïve cattle (*lane 5*)

23 kDa (Fig. 1b) and were as follows: 260, 250, 150, 130, 120, 80, 67, 60, 55, 35, and 23 kDa. All protein bands reacted with positive *E. granulosus* sera ($n=10$) except the 260- and 35-kDa bands. Furthermore, all but the 260-, 60-, and 35-kDa protein bands reacted with a *T. saginata*-positive sera ($n=10$).

SDS-PAGE and immunoblot of *T. hydatigena* cyst antigen

The protein bands of *T. hydatigena* ranged from 290 to 14 kDa (Fig. 1c). These were as follows: 290, 270, 260, 150, 130, 80, 67, 55, 35, 23, and 14 kDa. All protein bands reacted with *T. saginata*-positive sera ($n=10$) except the 290- and 14-kDa protein bands. Furthermore, all protein bands reacted with positive *E. granulosus* sera ($n=10$) except the 290-, 270-, 260-, and 14-kDa bands.

Discussion

Protection of human health against zoonoses is one of the key objectives of consumer-relevant legislation in the European Community. In its opinion on zoonoses adopted on 12 April 2000, the Scientific Committee on Veterinary Measures Relating to Public Health considered that the measures in place for the control of food-borne zoonotic infections were insufficient (SCVPH 2000). It further considered that the epidemiological data collected by Member States were incomplete and not fully comparable. As a consequence, the Scientific Committee recommended improved monitoring arrangements and identified risk management options. In order to improve this situation, the European Parliament and Council announced Directive 2003/99EC on the monitoring of zoonoses and zoonotic agents. The purpose of this Directive is to ensure that zoonoses and zoonotic agents are properly monitored and that food-borne outbreaks are subjected to proper epidemiological investigation. *T. saginata* cysticercosis was identified as one of a number of relevant pathogens which should be included in monitoring systems.

Application of serological methods for the detection of cysticercosis in carcasses would greatly improve the efficacy of efforts to eliminate human taeniosis and thus bovine cysticercosis. As the meat safety system, which is based only on conventional postmortem inspection at slaughterhouse, is apparently of relatively low efficacy both for preserving public health and for epidemiological studies, alternative methods should be studied thoroughly so that they can be used to replace conventional meat inspection methods.

The presence of shared antigenic components between *Taenia* and *Echinococcus* species, which belong to the same family (Taeniidae), was clear throughout the present study, confirming many published scientific papers (Oliveira et al. 2010; Gonzalez et al. 2007; Hancock et al. 2004). Eight proteins from *E. granulosus* were recognized to be immunoreactive with antibodies from animals experimentally infected with *T. saginata*. The extracted protein bands 35 and 260 kDa of *E. granulosus* that did not show immunoreaction, neither with anti-*E. granulosus* nor with *anti-T. saginata* cyst antibodies, might not be exposed to circulating immune cells and thus considered as "hidden" antigens. Antibody cross-reactivity of *T. saginata*- and *E. granulosus*-infected animals with *T. hydatigena* cyst antigen was also obvious. Nine extracted proteins from *T. hydatigena* cysts reacted positive with serum antibodies against *T. saginata* cysts and seven proteins with antibodies against *E. granulosus* cysts. Eight of the ten isolated *T. saginata* cyst antigens were recognized by *E. granulosus*-positive serum, but 14- and 18-kDa *T. saginata* protein bands showed no *E. granulosus* cross-reactivity and thus might be potential candidates for the development of a *T. saginata*-specific immunoassay. Such an assay is not only beneficial at the abattoir level but also on farm level for early identification of cattle herds at risk for beef tapeworm infections. Unfortunately, no *T. hydatigena*-positive serum was available to test cross-reactions between species of the genus *Taenia*. However, the 14-kDa band of *T. hydatigena* cysts was not recognized by *T. saginata*-positive serum. Therefore, 14-kDa *Taenia* proteins might be species specific and not result in false-positive serological tests. The same might be true for the 18-kDa band of *T. saginata*, to which a *T. hydatigena* equivalent was missing. However, even if there would be antibody cross-reactivity among different *Taenia* species infecting bovines as intermediate hosts, consideration has to be given whether false-positive results would be accepted to prevent infections of humans with the zoonotic beef tapeworm.

The major 67-kDa protein present in all tested cestode species could be used for future work on recombinant vaccine. Sera from cattle infected with the trematode *F. hepatica* did not recognize any of the protein bands of included cestode species so that cross-reactivity in a future *T. saginata* immunoassay is unlikely to occur. The same is suggested to be true for parasite-naïve cattle.

In conclusion, the results show that there is a wide range of antigenic similarity between the tested helminths. These similarities may provide the possibility to develop a vaccine against all taeniids affecting cattle. Two *T. saginata* protein bands were identified as potential serodiagnostic antigens. Additional research including sera from cattle infected with a substantial number of different *Taenia* species would be desirable to confirm presented results and gain further information on antigen–antibody interactions among cattle taeniids.

Acknowledgments The authors gratefully acknowledge the financial support of the German Academic Exchange Service (DAAD) under grant A/12/08212.

References

Abuseir S, Epe C, Doherr MG, Schnieder T, Nagel-Kohl U, Probst D, Kühne M (2010) Seroprevalence of *Taenia saginata* cysticercosis in the federal state of Lower Saxony in Germany. Berl Munch Tierarztl Wochenschr 123:392–396

Abuseir S, Epe C, Schnieder T, Klein G, Kühne M (2006) Visual diagnosis of *Taenia saginata* cysticercosis during meat inspection: is it unequivocal? Parasitol Res 99:405–409

Adwan G, Adwan K, Bdir S, Abuseir S (2013) Molecular characterization of *Echinococcus granulosus* isolated from sheep in Palestine. Exp Parasitol (in press)

Baumeister S, Schuh C, Dennis RD, Walther M, Pfister K, Geyer E (1995) Bovine cysticercosis: demonstration in experimentally infected calves of serum IgG antibodies reactive with neutral glycolipids of *Taenia saginata* and *T. crassiceps* metacestodes. Parasitol Res 81:18–25

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254

Casulli A, Manfredi MT, La Rosa G, Cerbo AR, Genchi C, Pozio E (2008) *Echinococcus ortleppi* and *E. granulosus* G1, G2 and G3 genotypes in Italian bovines. Vet Parasitol 155:168–172

Dennis RD, Baumeister S, Irmer G, Gasser RB, Geyer E (1993) Chromatographic and antigenic properties of *Echinococcus granulosus* hydatid cyst-derived glycolipids. Parasite Immunol 15:669–668

Dorny P, Vercammen F, Brandt F, Vansteenkiste W, Berkvens D, Geerts S (2000) Sero-epidemiological study of *Taenia saginata* cysticercosis in Belgian cattle. Vet Parasitol 88:43–49

Eichenberger RM, Stephan R, Deplazes P (2011) Increased sensitivity for the diagnosis of *Taenia saginata* cysticercus infection by additional heart examination compared to the EU-approved routine meat inspection. Food Control 22:989–992

EFSA (European Food Safety Authority) (2006) Opinion of the Scientific Panel on Biological Hazards (BIOHAZ) and of the Scientific Panel on Animal Health and Welfare (AHAW) on "Review of the Community Summary Report on Trends and Sources of Zoonosis, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004". EFSA Journal 4:403

EFSA (European Food Safety Authority) (2011) The European Union summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009. EFSA Journal 9:2090

Flávia de Assunção G, Gleyce AM, Heliana BO, Maria TN, Pacheco R, José RM, Julia MC (2010) Hydrophobic fraction of *Taenia saginata* metacestodes, rather than hydrophilic fraction, contains immunodominant markers for diagnosing human neurocysticercosis. Rev Soc Bras Med Trop 43:254–259

Geerts S, Kumar V, Ceulemans F, Mortelmans J (1981) Serodiagnosis of *Taenia saginata* cysticercosis in experimentally and naturally infected cattle by enzyme-linked immunosorbent assay. Res Vet Sci 30:288–293

Gonzalez LM, Ferrer E, Spickett A, Michael LM, Vatta AF, Gárate T, Harrison LJ, Parkhouse RM (2007) The *Taenia saginata* homologue of the major surface antigen of *Echinococcus* spp. is immunogenic and 97 % identical to its *Taenia solium* homologue. Parasitol Res 101:1541–1549

Hancock K, Pattabhi S, Greene RM, Yushak ML, Williams F, Khan A, Priest JW, Levine MZ, Tsang VCW (2004) Characterization and cloning of GP50, a *Taenia solium* antigen diagnostic for cysticercosis. Mol Biochem Parasitol 133:115–124

Hyeong-Kyu J, Keeseon SE (2009) Immunoblot patterns of *Taenia asiatica* taeniasis. Korean J Parasitol 47:73–77

Ishida MM, Rubinsky-Elefant G, Ferreira AW, Hoshino-Shimizu S, Vaz AJ (2003) Helminth antigens (*Taenia solium*, *Taenia crassiceps*, *Toxocara canis*, *Schistosoma mansoni* and *Echinococcus granulosus*) and cross-reactivities in human infections and immunized animals. Acta Trop 89:73–84

Minozzo JC, Gusso RLF, Castro EA, Lago O, Soccol VT (2002) Experimental bovine infection with *Taenia saginata* eggs: recovery rates and cysticerci location. Braz Arch Bio Technol 45:451–455

Oliveira HB, Machado GA, Mineo JR, Costa-Cruz JM (2010) *Taenia saginata* metacestode antigenic fractions without affinity to Concanavalin A are an important source of specific antigens for the diagnosis of human neurocysticercosis. Clin Vaccine Immunol 17:638–644

Onyango-Abuje JA, Hughes G, Opicha M, Nginyi KM, Rugutt MK, Wright SH, Harrison LJ (1996) Diagnosis of *Taenia saginata* cysticercosis in Kenyan cattle by antibody and antigen ELISA. Vet Parasitol 61:221–230

SCVPH (Scientific Committee on Veterinary Measures Relating to Public Health) (2000) Opinion on the control of taeniosis/cysticercosis in man and animals. http://ec.europa.eu/food/fs/sc/scv/out36_en.pdf. Accessed 20 December 2012

SCVPH (Scientific Committee on Veterinary Measures relating to Public Health) (2003) Opinion on revision of meat inspection in veal calves. http://ec.europa.eu/food/fs/sc/scv/out65_en.pdf. Accessed 20 December 2012

Tsang VC, Brand JA, Boyer AE (1989) An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J Infect Dis 159:50–59

Van Knapen F, Buys J (1985) Tapeworms in the Netherlands. Tijdschr Diergeneeskd 110:761–770