

## A REVIEW ON THE DIAGNOSIS INFECTION IN CATTLE OF *Schistosoma bovis*: CURRENT STATUS AND FUTURE PROSPECTS

### REVISÃO SOBRE DIAGNÓSTICO DE INFECÇÃO POR *Schistosoma bovis* EM BOVINOS: ESTADO ATUAL E PERSPECTIVAS PARA O FUTURO

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#### SUMMARY

Bovine schistosomiasis, caused by *Schistosoma bovis*, is a serious veterinary problem in many parts of the world. The current methods used for the diagnosis of the disease include clinical signs, pathological lesions, parasitological and serological techniques. As clinical signs and parasitological lesions caused by *S. bovis* are indistinguishable from those induced by other trematode parasites, confirmation of diagnosis by these methods is unreliable. Parasitological techniques used to demonstrate eggs of the parasite in fecal or tissue samples represent the most accurate method for detection of an active *S. bovis* infection. The tissue of choice for detection of *S. bovis* infection is the liver because of the visible macroscopic lesion that can be seen in that organ and the rapid detection of the parasite eggs under the microscope using crush smears. The serological techniques used for diagnosis of the disease do not necessarily identify an active infection. In addition, some of the positive reactions are non specific. However, serology is useful to identify previous infection in epidemiologic study. The ELISA has been recently validated for the diagnosis of bovine schistosomiasis and will probably replace the other serological tests. The

immunoblotting technique has been proven satisfactory to detect antibodies to defined and recombinant schistosome antigen vaccines. Nucleic acid hybridization techniques have been described for the study of schistosome species-specific identification. However, these molecular techniques have not yet revolutionarized diagnosis of schistosomiasis. These techniques will probably serve as the basis for future diagnostic tests.

**Key words:** *Schistosoma bovis*, bovine schistosomiasis, diagnosis, cattle.

#### RESUMO

Esquistossomose bovina, causada pelo parasita *Schistosoma bovis*, é um problema muito sério para a Veterinária em muitas partes do mundo. Os métodos atuais utilizados para o diagnóstico da doença incluem sinais clínicos, lesões patológicas, e técnicas de parasitologia e sorologia. Como os sinais clínicos e as lesões parasitológicas causadas pelo *S. bovis* são distingüíveis em relação às lesões induzidas por outros trematódeos, a confirmação do diagnóstico por estes métodos é questionável. Técnicas de parasitologia

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utilizadas para demonstrar ovos do parasita em amostras fecais ou teciduais representam os métodos mais acurados para detecção de uma infecção ativa pelo *S. bovis*. O tecido de preferência para detecção da infecção causada pelo *S. bovis* é o fígado devido à visível lesão macroscópica que pode ser vista neste órgão e da rápida detecção dos ovos do parasita usando "crush smears" e visualização microscópica. As técnicas de sorologia utilizadas para o diagnóstico desta doença não identificam necessariamente uma infecção ativa. Adicionalmente, algumas reações positivas não são específicas. Entretanto, sorologia é válida para identificar infecções prévias em um estudo epidemiológico. O teste de ELISA tem sido desenvolvido recentemente para o diagnóstico de equistossomose bovina e provavelmente substituirá os outros testes sorológicos. A técnica de "immunoblotting" tem sido satisfatoriamente aprovada para a detecção de anticorpos e vacinas recombinantes do antígeno do esquistossoma. Técnicas de hibridização do ácido nucleico tem sido descritas para o estudo de identificação de especificidade do schistosoma. Entretanto, estas técnicas moleculares ainda não revolucionaram o diagnóstico da esquistossomose. Provavelmente, estas técnicas ainda serão base para os testes de diagnóstico do futuro.

**Palavras-chave:** *Schistosoma bovis*, esquistossomose bovina, diagnóstico, bovinos.

## INTRODUCTION

Schistosomiasis is a chronic debilitating infection of humans and animals caused by different species of schistosomes and hence the disease is of public health importance. *Schistosoma bovis*, the cause of bovine schistosomiasis, is one of the major veterinary problems in many Mediterranean and African countries (HUSSIEN, 1973; BUSHARA et al., 1978; MAJID et al., 1980; ARADAIB, 1988). Bovine schistosomiasis may cause morbidity and mortality among susceptible ruminants (HUSSIEN, 1968; MALIK, 1969; HUSSIEN, 1973; ARADAIB & ABBAS; 1985; ARADAIB et al., 1994e). In cattle, the economic importance of the disease is mainly attributed to morbidity, mortality at the age of 6-30 months, liver condemnation, reduced productivity and poor subsequent reproductive performance (McCAULEY et al., 1984). Most of the research on the diagnosis of schistosomiasis is directed towards the species of medical importance and little is known in relation to the species of veterinary importance, the *S. bovis*. Current diagnosis of *S. bovis* infection by traditional methods includes evaluation of clinical signs, pathological lesions, parasitological and serological techniques. As clinical signs caused by *S. bovis* are similar to those produced by other trematode parasites,

confirmation of *S. bovis* infection under field condition by these method is unreliable (GORAISH et al., 1988, ARADAIB et al., 1993). At necropsy, the presence of adult worms of the parasite in the mesenteric vessels or demonstration of the parasite eggs in crush smears from infected tissues represents the most accurate diagnosis (OSMAN, 1984). Parasitological diagnosis by finding eggs in a fecal sample or biopsy specimen will remain the only definitive diagnostic method for detection of an active *S. bovis* infection in a living individual. The major thrust of the current research conducted in our laboratory is directed towards the improvement of the existing techniques used for diagnosis of *S. bovis* infection in cattle. The development of a rapid sensitive specific and inexpensive method for diagnosis of the disease would greatly facilitate clinical disease investigations, epidemiological investigation, treatment of the infected animals and would enhance vaccination and control programs. This report is intended to summarize the previous and the current methods used for diagnosis of bovine schistosomiasis. Future prospect for diagnostic aspect of the disease is discussed.

## DIAGNOSIS OF *S bovis* INFECTION

### 1. Clinical signs

Zebu cattle infected with *S. bovis* develop a syndrome characterized by weight loss, poor weight gain, diarrhoea, loss of appetite, roughness of the skin, and pale mucous membranes (SAAD et al., 1980; ARADAIB et al., 1994a). These signs are usually observed by 6-7 weeks after exposure to the infective stage, the cercaria. The severity of these signs increases between the 7th and the 9th week, where the fecal egg counts are highest (DARGIE, 1980). However, the clinical signs of the disease are unreliable as other trematodes parasites may produce similar clinical signs. The previously described clinical signs were observed in calves and goats experimentally infected with *S. bovis* and *Fasciola gigantica*, respectively (GORAISH et al., 1988; ARADAIB, 1993, ARADAIB et al., 1994a).

### 2. Postmortem findings

At necropsy, *S. bovis* infection can be diagnosed by finding thousands of visible adult worms in the mesenteric vessels. Infected livers are diagnosed on the basis of the presence of macroscopic lesions of schistosomiasis visible as white-gray foci under the liver capsule and within the substance of the liver (OSMAN, 1984). However, in certain instances few lesions may be present and may not be detected and hence crush smears made from those livers are necessary for demonstration of *S. bovis* eggs to confirm the diagnosis. In a living animal,

however, the pathology of *S. bovis* is related to fecal egg excretion and this finding renders parasitological examination by finding eggs of the parasite in fecal samples an interesting alternative to biopsy specimens (SAAD et al., 1980; DARGIE, 1980; ARADAIB, 1988).

### 3. Parasitological techniques

Parasitological diagnosis of *S. bovis* infection by demonstration of the parasite eggs in fecal sample is possible by 5-8 weeks post infection, depending on the level of the infection (ARADAIB, 1994d). The fecal egg excretion remains high during the 2 months following patency after which it declines to a few number of eggs per gram of feces (SAAD et al., 1980; ARADAIB, 1988). Definitive diagnosis of an active *S. bovis* infection can be made only by detecting eggs of the parasite in feces or biopsy specimen of the infected animal. The routine methods used for parasitological diagnosis include; fecal smear, filtration method, sedimentation method, rectal and liver biopsy and miracidial hatching test. The most commonly used method for detection of fecal egg excretion under field condition is the sedimentation method. Briefly, five grams of feces are mixed with 20 times their volume of normal saline and the mixture allowed to settle in a glass urine flask. the supernatant is removed after half an hour and the sediment resuspended. This step is repeated until the supernatant becomes clear. A drop of the final sediment is placed in a slide with a cover slip and examined under the microscope. *S. bovis* egg has a prominent central bulge and terminal spine and measures about 130-260 um in length (PICHFORD and VISSER, 1975). This method can be used qualitatively for detection of eggs in clinical practice. The filtration method described by BUSHARA et al. (1978), however, can be used qualitatively or quantitatively for research in the field of epidemiology including drug trials and vaccination programs. The main advantage of parasitological diagnosis is the confidence with which the species causing the disease can be determined and the confidence with which chemotherapy can be initiated (JORDAN & WEBBE, 1982). In addition, the state of the eggs reflects the stage to which the disease has advanced, where in chronic infections the eggs are likely to be dead and calcified (OSMAN et al., 1984). Parasitological techniques have reliably served both in clinical diagnosis and epidemiological surveys of schistosomiasis but they do suffer from certain weaknesses. Chronically infected cattle develop immunity against infection and egg production is greatly suppressed (DARGIE, 1980; ARADAIB, 1993). The implication of this is that fecal examinations only succeed in detecting eggs in a minority of cases. Since disease intensity is related to fecal egg excretion in active infections, the overall effect is to underestimate both the intensity and prevalence during an epidemiological survey and those individuals with low infection levels or chronic

infection are likely to be missed diagnosed (MURARE, 1983). In addition, Parasitological tests depend for their sensitivity on examining large samples or repeated examination of several small samples. It has been reported that even repeated fecal examination of over 3 years was unreliable for measurement of incidence of infection with *S. japonicum* (LEWERT et al., 1984). Moreover, parasitological methods, despite the problems associated with their use, will remain important diagnostic tools by which an active schistosome infection can be detected since there is yet no other procedure by which unequivocal evidence of schistosomiasis in a living animal can be obtained. It is worth mentioning that *S. bovis* is not a zoonotic trematode parasite. However, detection of *S. bovis* eggs in a human stool sample is not surprising. This is because in certain parts of Africa human consumption of uncooked infected sheep liver is not uncommon.

Because the sensitivity of parasitological tests is variable, depending on the level of egg excretion and effective sample size used, there is an increasing emphasis on developing serological techniques for diagnosis of bovine schistosomiasis (ARADAIB, 1988).

### 4. Serological techniques

Serology does not necessarily identify an active *S. bovis* infection. However, it is useful to determine past infection in a seroepidemiological survey. Several serodiagnostic techniques have been validated for the diagnosis of schistosomiasis. The main bulk of the work, so far, has been on the species infecting humans rather than on *S. bovis*, the species of veterinary importance. The serological techniques applied for diagnosis of *S. bovis* include intradermal test for immediate and delayed hypersensitivity; serological tests based on agglutination reaction, e.g. indirect hemagglutination test (IHA); the precipitation tests such as circumoval precipitin test (COPT), Cercarial Hullen reaction (CHR), agar gel immunodiffusion (AGID); the complement fixation test (CFT); and the indirect labeled antibody technique such as the indirect immunofluorescence antibody technique (IFAT); the enzyme linked immunosorbent assay (ELISA) and immunoblotting such as western blots.

#### *i. Immediate and delayed hypersensitivity test*

The test has not been described for the diagnosis of bovine schistosomiasis on a practical scale. Adult worm antigen (AWA) has proven satisfactory for detection of immediate and delayed skin test. However, cercarial antigen was found to be of little value because of the large number of the false positive reaction (SAAD, personal communication). A positive result is indicated by increased thickness of the skin at the site of the injection.

## ii. Circumoval precipitin test (COPT)

This test requires preparation of viable eggs. A positive result is indicated by the presence of globular or long chain type of precipitate around the eggs. The test was reported to be sensitive and specific by several workers (JIAFU et al., 1984).

## iii. Cercarian Hullen reaction

The test is simple rapid and sensitive. A positive reaction is indicated by formation of an envelope or a precercarial sheath around the cercariae. The test is simple and sensitive. However, it requires live infectious cercariae which limits its use under field condition as cercariae are only available in a laboratory maintaining infected snails. The test has proven satisfactory for diagnosis of *S. bovis*. However, most of the work constitute unpublished data.

## iv. Agar-gel immunodiffusion test (AGID)

The method adopted was basically that described by OUCHTERLONEY (1958) with the modification of ARADAIB et al. (1993). Precipitin lines were seen when *S. bovis* adult worm antigen was tested against hyperimmune sera from *S. bovis* infected rabbit and mice (HAROUN, 1973; MURARE, 1983). Precipitin lines were also observed with sera from calves experimentally infected or immunized with *S. bovis* adult worm extracts or whole-egg antigen (ARADAIB et al., 1993; ARADAIB et al., 1994a; ARADAIB et al., 1994b). Cross reactions in the AGID between whole-egg, cercarial or adult worm antigens were also observed (ARADAIB, 1988; ARADAIB, 1992). No precipitin lines were observed with sera from calves experimentally immunized with irradiated *S. bovis* schistosomula (ARADAIB et al., 1994b).

## v. Indirect fluorescence antibody technique (IFAT)

The test was first described by HUSSIEN (1972). A positive result is indicated by presence of specific fluorescence. The IFAT was found to be 95% sensitive for the diagnosis of *S. mansoni* using adult worm antigen. The specificity decreased very sharply when cercarial antigen was used due to large number of false positive reaction (SCHINIKI et al., 1976). HUSSIEN (1972) applied the test for serodiagnosis of *S. bovis* infection in calves and suggested that partially purified *S. bovis* adult worm may be used as antigen for diagnosis of bovine schistosomiasis. However, the technique is tedious and liable to error because it is rather subjectively scored. In addition, a positive result may be obtained from an individual who had been exposed to avian or other non human schistosomes (JORDAN & WEBBE, 1982).

## vi. Complement fixation test (CFT)

The CFT requires the use of sensitized RBCs of sheep. A positive result is indicated by absence of RBCS hemolysis due to fixation of the complement by the antigen-antibody reaction, whereas hemolysis indicate a negative test. The test is sensitive and has a very high specificity in the diagnosis of human schistosomiasis using adult worm antigen. The sensitivity and the specificity of the test were found to be 69% and 100% respectively. A large number of false positive reactions were obtained when using cercarial antigen (HUSSIEN, 1972). HAROUN (1973) reported the test to be of great value for diagnosis of bovine schistosomiasis using adult worm antigen. However, the test is complicated, highly delicate technique and is not suitable for field operation.

## vii. Enzyme linked immunosorbent assay (ELISA)

The microplate modification version of ELISA was first described by VOLLER et al. (1976). The potential of ELISA for diagnosis of a variety of parasitic infections has been investigated, and ELISA was found to be more sensitive than earlier serological tests. ELISA is more likely to be used in developing countries since minimal laboratory equipments are required. In addition, ELISA can be used as a superior diagnostic alternative to other serological test because it is a simple, rapid, inexpensive and sensitive test. One of the major problems in the application of ELISA for diagnosis of bovine schistosomiasis is the availability of a suitable and specific antigens that can be used to avoid or to minimize false positive results due to cross reactions with other helminth infections. HULDT et al., (1975) were the first authors to detect antibodies to human schistosomes by ELISA and ARADAIB (1988) was the first author to detect antibodies to bovine schistosomiasis. Both authors noted the potential of ELISA for seroepidemiological study of schistosomiasis. Most investigators have used crude or partially purified antigens (MURARE, 1983; ARADAIB, 1988; ARADAIB et al., 1994a; ARADAIB et al., 1994b). Application of soluble egg antigens (SEA) had commonly been reported to yield a better results than adult worm antigens (AWA), irrespective of their purity (VOLLER et al., 1976; MOTT and DIXON, 1982). ARADAIB (1988) observed cross reactions between *S. bovis* AWA and SEA in the ELISA with characteristically lower absorbance values to heterologous antigen. A variety of different antigenic preparations for the diagnosis of human schistosomiasis were described. However, little work has been carried out in relation to the species of veterinary importance. A purified *S. japonicum* SEA increased the specificity and the sensitivity of the ELISA to 100% to 98% (MATSUDA et al., 1984). In a previous report, we have validated the potential of ELISA for the diagnosis of *S.*

*bovis* infection using defined and recombinant *S. mansoni* 28.000 daltons protein antigens. Unfortunately, this antigen did not yield any diagnostic value for detection of heterologous infection with *S. bovis* (ARADAIB et al., 1994c). The structural differences within the immunologically essential regions of schistosome 28.000 dalton proteins are thought to be responsible for this interspecies variation (TROTTEIN et al., 1992). Experimental immunization of calves with *S. bovis* 28.000 daltons protein antigen induced specific IgG antibody responses which were detected by ELISA and immunoblotting technique at the time of challenge. There was association between antibody production and protection (BUSHARA et al., 1993).

#### viii. Immunoblotting technique

This technique has been recently introduced for detection of *S. bovis* defined or recombinant antigens against specific IgG antibodies. Western blots are first incubated with antisera at room temperature and then with labeled antibody at room temperature and finally a substrate is added for visualization of the antigen-antibody reaction at the expected weight of the protein using molecular weight marker. The technique is highly sensitive and specific. Using western blots, sera from calves vaccinated with defined or recombinant *S. bovis* or *S. mansoni* 28 KD proteins recognized protein bands at a molecular weight of 28 KD by 3 weeks post vaccination. The technique is useful to study the immune response of animals to schistosome vaccines as well as correlation between antibody production and protection (ARADAIB, 1988; BUSHARA et al., 1993).

The serological techniques mentioned previously, despite their advantage in detecting *S. bovis* infected individuals, are complicated by cross reactions between other trematode parasites and within different species of schistosomes. To address these problems, molecular diagnostic techniques have been developed.

### 5. Molecular diagnostic techniques

The gene coding for schistosome glutathione-S-transferase (GST) has been identified, cloned and sequenced (BALLOUL et al., 1987a and 1987b). Nucleic acid hybridization techniques using Complementary DNA (cDNA) probes have been developed for detection of nucleic acid sequence of schistosome GSTs. Primers-directed reverse transcriptase (RT) polymerase chain reaction (PCR) for sequencing of schistosome GSTs was also described (TROTTEIN et al., 1992). All these molecular studies have been carried out in an attempt to produce an effective broad spectrum antischistosome vaccine. However, no work has yet been conducted in molecular diagnosis of schistosomiasis on a practical scale. These molecular

diagnostic techniques probably will provide the basis for future diagnostic tests.

### CONCLUSION

Parasitological techniques used to demonstrate *S. bovis* eggs in a fecal or a tissue sample represent the most accurate diagnosis for bovine schistosomiasis. Serodiagnostic tests are useful to identify a previous infection in epidemiological studies. Molecular diagnostic techniques including cDNA probes and PCR technology may improve the existing diagnostic techniques used for species-specific identification of schistosome. These molecular diagnostic techniques will provide the basis for better understanding of the epidemiology of the disease. The development of a rapid, sensitive and specific test will facilitate clinical disease investigation, herd health monitoring and enhance vaccination and control programs.

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