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A study of nasal and visceral schistosomosis in cattle slaughtered at an abattoir in Bangalore, South India

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Summary
The authors studied the incidence of nasal and visceral schistosomosis in 300 male cattle that were slaughtered in 2001 at the Karnataka Meat and Poultry Marketing Corporation Limited abattoir in Bangalore, South India. The nasal and intestinal scrapings and their contents were screened for eggs and the nasal and visceral cuttings were examined for worms. Eggs of *Schistosoma nasale* and *S. spindale* were observed in 197 and 151 samples and worms in 218 and 204 carcasses respectively. A mild infection of *S. nasale* was observed in 129 animals and a mild infection of *S. spindale* in 124 animals, a moderate infection of *S. nasale* was found in 77 animals and a moderate infection of *S. spindale* in six animals. Twelve animals were heavily infected with *S. nasale* and 14 cattle were heavily infected with *S. spindale*. Mixed infections of both species occurred in 163 animals. The level of infection was determined by the eggs per gram method and was found to be in the range of 1 to 310 eggs per gram for *S. nasale* and 1 to 201 for *S. spindale*, this was a higher result than the eggs per cubic centimetre method, which indicated lower egg counts. Worms were recovered from 218 nasal and 204 visceral cuttings. It was concluded that the incidence of nasal and visceral schistosomosis in cattle in this region is under-diagnosed and the carrier status of these animals in the subclinical state is potentially high.

Keywords
Abattoir – Diagnosis – Incidence – India – Nasal – Schistosomosis – Visceral schistosomosis.

Introduction

Schistosomosis is a snail-borne trematode infection of man, domestic animals and wild animals in different parts of Asia and Africa. Schistosomosis is now well recognised as the fifth major helminthosis of domestic animals in the sub-continent. The species which commonly occur in India are *Schistosoma nasale* and *S. spindale* in cattle, *S. indicum* in equines and sheep and *S. incognitum* in pigs.

In the epidemiology of schistosomosis in cattle, the prevalence of infection and the incidence of clinical disease must be differentiated. Reports on the prevalence of the infection have been principally based on samples collected during field surveys or clinical cases and, except for two studies involving the systematic screening of slaughtered animals (5, 6), research to assess the actual status of infection and the incidence of the disease has not been carried out.

Schistosoma nasale and *S. spindale* cause several clinical and pathological manifestations even in moderate infections. These blood flukes adversely affect health and productivity and serious outbreaks of disease caused by these two species have been reported (1, 2).

In Karnataka state, to which Bangalore belongs, some studies on the epidemiology, diagnosis and control of schistosomosis, based on clinical cases or in restricted regions, were conducted in the later part of the 20th Century (7, 9, 11). The present study was undertaken to observe the actual status of schistosomosis in cattle in slaughtered animals in Bangalore.

Materials and methods

To observe the incidence of schistosomosis, as a prelude to detailed examination of infected organs, nasal scrapings and small and large intestinal scrapings were taken from 300 animals slaughtered in the year 2001 at the Karnataka Meat and Poultry Marketing Corporation Limited abattoir in Bangalore. The animals slaughtered were all male, since the law prohibits the slaughter of female animals. The faecal samples from the large intestines were screened for the eggs of *S. spindale* by using a sedimentation technique and subsequently the nasal and intestinal scrapings were also screened for eggs. The scrapings, which were obtained when the carcasses were dressed, were screened by two methods, i.e. the eggs per cubic centimetre (EPCC) method and the eggs per gram (EPG) method (10).

Eggs per cubic centimetre method

One ml of nasal scrapings and 1 ml of intestinal scrapings were collected with a vaulkmans scoop. These scrapings were each mixed with 9 ml of 10% potassium hydroxide in a test tube. After mixing, the tubes were kept at room temperature for 10 to 20 min., the lower contents were then spread over slides and the eggs were counted under low magnification to estimate the EPCC.

Eggs per gram method

One ml of nasal scrapings and 1 ml of intestinal scrapings, collected by means of a vaulkmans scoop, were each mixed with 5 ml of 10% potassium hydroxide in wide-bored pipettes and transferred to a test tube for heating. After heating, the contents were centrifuged at 3,000 rpm for two to three minutes. The supernatant fluid was carefully poured off, retaining the sediment with a few drops of fluid. After mixing it thoroughly, the sediment was drawn-off completely or in portions, depending upon the quantity, with the aid of a long glass dropper and transferred to a slide, which was then covered by a coverslip. All the eggs were counted under low magnification. The total number of eggs in the entire sediment was taken to be the number of EPG per scraping.

Collection of worms

Material for further studies was collected during evisceration and dressing from the same animals from which nasal and intestinal scrapings were taken. The entire cranial part of one nasal cavity was opened by two horizontal incisions inside the nasomaxillary incisure, one above the nasal process of the incisive bone, the other one under the nasal bone. Samples were taken from the lateral wall of the cavity, the conchae and the underlying part of the nasal septum. The nasal samples were cut into small pieces and were immersed in large glass bowls containing normal saline and left undisturbed for four to five hours. The cuttings were then taken out, the normal saline was filtered through a black muslin cloth into another bowl and the cloth was inverted into a Petri dish containing normal saline. When present, *S. nasale* were observed directly or under a stereo microscope and worm counts were made. The mesenteries of both the large and small intestines were cut into small pieces and were immersed in large glass bowls containing normal saline and left undisturbed for four to five hours to recover *S. spindale*. The intensity of infection was considered to be mild when 1 to 20 worm pairs were recovered (schistosomes always occur in pairs as the female worm is carried by the male in the gynaecophoric canal), moderate when 20 to 100 worm pairs were found and heavy when more than 100 worm pairs were observed.

The data were analysed using the Man Whitney non parametric test (17).

Results

The incidence of infection based on the detection of *S. nasale* and *S. spindale* eggs and worms in the material collected from the slaughtered cattle is presented in Table I. The incidence of nasal and visceral schistosomosis based on the detection of eggs was 65.6% and 50.3% respectively and 34.3% and 49.6% were negative for *S. nasale* and *S. spindale* respectively.

Table I
Incidence of schistosomes based on the detection of eggs and worm pairs from slaughtered cattle at Bangalore

Results	*Schistosoma nasale*		*Schistosoma spindale*	
	Eggs	Worm pairs	Eggs	Worm pairs
Number of samples tested	300	300	300	300
Number positive	197	218	151	204
Percent positive	65.6	72.6	50.3	68
Number negative	103	82	149	96
Percent negative	34.3	27.3	49.6	32

Out of 300 samples screened for worms, 218 (72.6%) and 204 (68%) were positive and 82 (27.3%) and 96 (32%) were negative for *S. nasale* and *S. spindale* respectively.

The intensities of infection with *S. nasale* and *S. spindale* were determined based on the number of worm pairs obtained from the nasal cuttings or visceral cuttings (Table II). It was observed that 129 animals had a mild infection of *S. nasale*, 77 a moderate infection and 12 a heavy infection; 124 cattle had a mild infection of *S. spindale*, 6 cattle had a moderate infection and 14 a heavy infection. The highest recorded infection of *S. nasale* was 424 worm pairs and the highest infection of *S. spindale* was 508 worm pairs. Mixed infections with both species of *Schistosoma* were observed in 163 animals.

Table II
Intensity of infection of the two *Schistosoma* species in cattle based on the number of worm pairs obtained from nasal and visceral cuttings

Intensity	Worm pairs	Number of cattle infected with	
		Schistosoma nasale	*Schistosoma spindale*
Mild	1 - 20	129	124
Moderate	20 -100	77	6
High	>100	12	14

The level of infection was estimated by two different techniques: the EPCC method and the EPG method. The EPCC method produced results that ranged from 1 to 140 eggs of *S. nasale* and 1 to 96 eggs of *S. spindale*. The EPG method had a higher count, ranging from 1 to 310 eggs of *S. nasale* and 1 to 201 eggs of *S. spindale*. The results of statistical analysis are presented in Table III. The difference in means were significant at $P \leq 0.05$ level.

Table III
Comparison of the eggs per cubic centimetre (EPCC) and eggs per gram (EPG) methods for detecting *Schistosoma nasale* and *S. spindale* ova

Species	EPCC (mean ± C.I. 95%)	EPG (mean ± C.I. 95%)
Schistosoma nasale	41.8 ±10.9	61.1 ±14.0
Schistosoma spindale	29.7 ± 6.5	45.7 ± 8.5

C.I.: confidence interval

Discussion

Nasal and visceral schistosomosis, caused, by *S. nasale* and *S. spindale* respectively, are common infections of cattle in the Asian sub-continent and adjacent countries. They arouse considerable interest, as these infections of cattle and buffaloes, caused by blood flukes, are endemic and many foci of infection exist. Schistosomosis in bovines is known to cause severe morbidity in animals. Detection of subclinical schistosomosis in live animals is difficult, since the affected animals do not show obvious signs of disease and animals act as carriers and continue the chain of transmission. Nevertheless, it is important to detect schistosomosis in live animals so that they can be treated in order to minimize morbidity and economic losses. *Schistosoma spindale* infection is under-diagnosed, as the eggs in the faecal samples of affected animals are usually masked by a high mucus content and the eggs in faeces also easily hatch on contact with water and hence they are often missed.

As previous studies on the incidence and prevalence of nasal and visceral schistosomosis in Karnataka state have been based solely on the examination of nasal scrapings or faecal samples from live animals, they have underestimated the number of cases of infection. The present study, by examining the infection in slaughtered animals, provides a much clearer idea of the actual status of schistosomosis in the region. In the districts of Dharwar, Belgaum and North Kanara in Karnataka state, prevalence rates of 10%-15% infection for *S. nasale* in cattle have been reported (13) based on detection of *S. nasale* eggs in nasal washings and clinical signs such as a snoring sound, cauliflower-like growths, etc. However, the present study revealed a high incidence of 69.16% (including subclinical and carrier animals). Epizootiological studies on nasal schistosomosis in bovines in the Bangalore and Mandya districts of Karnataka state (11) indicated that old and female animals were more affected and that based on clinical signs and the detection of eggs in nasal discharge there was no difference in prevalence between breeds. In the present study, observations were carried out on slaughtered male animals which included nine- to eleven-year-old bulls and bullocks. Previous reports (3) have observed a high incidence of schistosomosis in older animals (44%) and in the present study the incidence among older animals was even higher (69.16%). Other previous findings, such as the incidence of 60.3% in non-descript bullocks and 68.87% in Hallikar bullocks of *S. nasale* infection in Andhra Pradesh (14) and observations that males were more susceptible than females based on the detection of eggs in nasal washings, corroborate the findings of the present study.

A study of *S. nasale* conducted at a slaughterhouse in Sri Lanka (5) revealed an overall percent prevalence of 12.6% based on the detection of eggs and cauliflower-like growths and lesions at the time of slaughter. In the present study a much higher rate of incidence of 69.16% based on detection of eggs and worms was observed. This could be attributed to the fact that in this study observations of worms were also made in the nasal cuttings. Therefore it is apparent that the actual incidence cannot be determined solely by examining clinical material or lesions.

In a collection of more than 500 *S. spindale* worms from a bullock, a higher population of male worms was reported from the portal veins, giving a sex ratio of 16:1 (16), however in the present study, more male worms than female worms were collected from the mesenteric veins, with a sex ratio of 12:1 for *S. spindale*. The incidence of *S. spindale* in Bangladesh, based on the detection of worms from visceral organs, indicated a lower incidence of 37% (8) when compared to the incidence rate of 59.16% in the present study. A previous report by some of the authors, based on examination of faecal samples by sieving and sedimentation techniques, reported an incidence rate of *S. spindale* infection of 4.27% in cattle aged over eight years in the Mysore and Mandya districts of Karnataka (7). The infection incidence rate observed in the present study, which was based on the detection of eggs from intestinal scrapings and worms from the mesenteric veins, was 59.16%. This is a much higher incidence than observed in the above study, indicating that the actual incidence in the previous study was probably considerably higher, but that the techniques used did not detect infection in subclinical animals. Similarly, a previous study, based on faecal examinations, reported prevalences of 0.41% and 0.1% of *S. spindale* in the Mysore and Mandya districts in Karnataka state (12), but the present study, based on the examination of intestinal scrapings for the presence of eggs and worms, indicated a higher percentage of infection of 59.16% for *S. spindale*. This strongly indicates that the incidence can be under-diagnosed when based solely on faecal examination, as the sensitivity of diagnosis of this method is very low. The mesenteric veins of 901 cattle examined for the presence of *S. spindale* at a slaughterhouse in Sri Lanka revealed an overall percent of infection of 31.2% (6). The present study revealed an overall infection rate of 68% based on the detection of *S. spindale* in the mesenteric veins of 300 animals, indicating that the disease is much more prevalent in and around Bangalore, where there seems to be a strong focus of infection.

In a previous study, a mixed infection was observed in five out of twenty cattle in natural infections of *S. nasale* and *S. spindale* (15). In the present study, a larger number of cattle, 168 out of 300, were infected with both *S. nasale* and *S. spindale*. In another study, it was reported that out of 80% of cattle suffering from nasal schistosomosis in Andhra Pradesh, 50% of the animals were positive for eggs of *S. spindale* (4). This correlates with the present study in which more than 50% of the cattle harboured both species of worms and were also positive for eggs. The present study reported no cases of schistosomosis in cattle reared at farms in Bangalore. Although cattle on these farms had been reared in confinement, the absence of even an isolated case of schistosomosis highlighted the fact that proper management practices alone can minimize the disease.

The EPG method was found to be more accurate than the EPCC method in egg counting, although the procedure of weighing mucus in the EPG method was time consuming. These observations were in agreement with another report on *S. nasale* (10), but in this study the usefulness of the EPG method over EPCC method was also established with *S. spindale* wherein the procedure was followed with intestinal scrapings. Hence the level of infection can be reliably detected by the EPG method for both *S. nasale* and *S. spindale* infections.

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Étude de la schistosomiase nasale et intestinale chez des bovins dans un abattoir de Bangalore (Inde méridionale)

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Résumé

Les auteurs ont étudié l'incidence de la schistosomiase nasale et intestinale chez 300 bovins mâles tués en 2001 dans l'abattoir de la société Karnataka Meat and Poultry Marketing Corporation Limited à Bangalore (Inde méridionale). Des biopsies par grattage de la muqueuse nasale et intestinale ont été effectuées pour la mise en évidence d'œufs. Par ailleurs, une recherche d'éléments parasitaires a été réalisée sur des coupes des muqueuses nasale et intestinale. Des œufs de

Schistosoma nasale et de *S. spindale* ont été observés respectivement dans 197 et 151 échantillons. Des vers de ces deux espèces respectives étaient présents dans 218 et 204 cadavres. On a relevé une infection discrète à *S. nasale* chez 129 animaux et à *S. spindale* sur 124 cadavres. Par ailleurs, une infection modérée à *S. nasale* et à *S. spindale* a été constatée respectivement chez 77 animaux et sur 6 cadavres. Douze animaux avaient été fortement infectés par *S. nasale*. Une forte contamination par *S. spindale* a été relevée chez 14 bovins. Des infections causées simultanément par les deux espèces ont été observées chez 163 animaux. Le niveau d'infection, déterminé en fonction du nombre d'œufs par gramme, variait de 1 à 310 œufs par gramme pour *S. nasale* et de 1 à 201 œufs par gramme pour *S. spindale*. Cette valeur dépassait celle obtenue par le dénombrement des œufs par centimètre cube. La présence de vers a été constatée sur 218 coupes de la muqueuse nasale et 204 coupes de la muqueuse intestinale. Cette étude a permis de conclure que l'incidence de schistosomiase nasale et intestinale était sous-diagnostiquée chez les bovins de la région et qu'un nombre important de ces animaux pouvaient être des porteurs subcliniques de la parasitose.

Mots-clés
Abattoir – Diagnostic – Incidence – Inde– Schistosomiase intestinale– Schistosomiase nasale.

Estudio sobre la esquistosomosis nasal y visceral del ganado faenado en un matadero de Bangalore, en el sur de India

S. Sumanth, P.E. D'Souza & M.S. Jagannath

Resumen
Los autores estudiaron la incidencia de la esquistosomosis nasal y visceral en 300 vacunos machos sacrificados en 2001 en el matadero de la Karnataka Meat and Poultry Marketing Corporation Limited, situado en Bangalore, en el sur de India. Se buscaron huevos en raspados nasales e intestinales y sus contenidos, así como helmintos en cortes nasales y viscerales. Se encontraron huevos de *Schistosoma nasale* y *S. spindale* en 197 y 151 muestras, y helmintos en 218 y 204 reses respectivamente. Se observaron infecciones ligeras de *S. nasale* en 129 animales y de *S. spindale* en 124, así como también infecciones moderadas de *S. nasale* en 77 reses y de S. spindale en 6 cadáveres. Doce animales sufrían infecciones graves de *S. nasale* y 14 de *S. spindale*. En 163 animales se detectaron infecciones mixtas de ambas especies. El grado de infección se determinó en función del número de huevos por gramo: éste oscilaba entre 1 y 310 huevos por gramo en el caso de *S. nasale* y entre 1 y 201 en el de *S. spindale*; estos resultados fueron superiores a los obtenidos con la medida del número de huevos por centímetro cúbico que indicó una cifra inferior. Se encontraron helmintos en 218 cortes nasales y 204 viscerales. Se llegó a la conclusión de que la incidencia de la esquistosomosis nasal y visceral en el ganado de la región ha sido infra-diagnosticada y que la condición de portadores de esos animales en estado asintomático es potencialmente elevada.

Palabras clave
Diagnóstico – Esquistosomosis nasal – Esquistosomosis nasal – Esquistosomosis visceral – Incidencia – India – Matadero.

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