Rhinosporidiosis is a benign, chronic, granulomatous fungal disease particularly of the mucous membranes of the nasal cavity and is caused by *Rhinosporidium seeberi* (Jungerman and Schwartzman, 1972; Emmons *et al.*, 1977). The disease is characterized by the formation of polypoid growths in the nasopharynx but also in the conjunctiva, larynx, oral cavity, rarely in the skin, genitals, rectum, bone and brain (Wright, 1907; Kral and Schwartzman, 1964; Prevost, Kreutner, Vallotton and Walker, 1980; Jimenez, Young and Hough, 1984). The polyps may occur as singular or multiple and generally involve only one nostril. Sometimes, the growth become sufficiently larger to occlude the nasal cavity and may cause stertorous breathing to the diseased subject (Jungerman and Schwartzman, 1972). The polypoid growth may be sessile or pedunculate and cauliflower-like, and may vary in size. They are friable, soft, pink to purplish red in colour and bleed easily after trauma. Thus blood stained mucopurulent nasal discharge may be observed at an early stage of the disease (Jubb and Kennedy, 1970; Blood, Henderson and Radostits, 1982).
lesions appear mostly in the nasal mucosa. Later
the polyps may extend from the nares to the pharynx
or lip and may attain a weight of 20 gms. The
close examination of the nasal cavity may reveal
minute, whitish spots which are the mature sporangia
of *Rhinosporidium seeberi* (Emmons et al., 1977).

The disease is endemic in India, Argentia and
Ceylon (Boyd, 1949; Smith, Jones and Hunt, 1972; Pal,
1931). However, sporadic cases have been described
from East Indies, Mexico, Iran, Cuba, South Africa,
Philippines, Australia, Italy, Brazil, England,
Scotland, Paraguay and the United States (Schlogal
and Curial, 1956; Laffont, 1961, Smith and
Frankson, 1963; Rippon, 1974; Marton, Pancratov
The prevalence of the disease is recorded more in
males both in man and animals (Rao, 1933; Conant, et al.,
1954; David, 1974; Owor and Wamukota, 1973). Further,
infection is considered more severe in man than animals
and dissemination of lesions has been observed in
human beings only (Dalta, 1965).

*Rhinosporidium seeberi* has a wide host range.
The infection has been recorded in the horse
(Zschokke, 1913; Sahai, 1938; Smith and Frankson,
Although the causative agent of rhinosporidiosis was described by Guillermo Sebber in 1900, *Rhinosporidium seeberi* as the fungus could be identified much later by Ashworth in 1922. The natural reservoir of the fungus could not be demonstrated so far; and it is, however, suggested that *R. seeberi* may perhaps occur as saprophyte in soil and water. A comprehensive and systemic study on the epidemiology of the disease failed to give any encouraging results as the fungus could not be isolated from fish, snails, water, manure and cyclops of the endemic regions (Reddy and Lakshminarayan, 1962). Further, the attempts to infect
fish and snails with contaminated water were also unsuccessful and futile. Interestingly, Grover (1970) was successful in showing maturation of sporangia and spores in TC 199 synthetic liquid medium when he placed biopsy material in this artificial media.

There appears to be little information on the clinico-epidemiology of nasal rhinosporidiosis in domesticated ruminants. Therefore, the present study was contemplated to find out the prevalence of Rhinosporidium seeberi in the nasal cavity of diseased animals. Attempts were also made to study the clinical spectrum of the disease. The material from the positive cases was tried to produce the disease experimentally in laboratory animals and also attempted to recover the causative agent on various cultural media.
MATERIALS AND METHODS

Source of clinical specimens:

The nasal exudates collected aseptically from the diseased animals constituted the material for this study. In all 139 nasal secretions, collected from 41 buffaloes, 39 cattle, 27 goats, 21 sheep and 11 camels, were investigated for the prevalence of nasal rhinosporidiosis. Of the 139 animals, 58 were males and 81 females. They belonged to all age groups ranging from less than 1 year to more than 12 years with maximum number in 3-5 years group (Table -16). The details regarding the age, sex, species of animal, clinical signs, duration of disease, history of trauma, source of water supply, provisional diagnosis, underlying disease, and treatment received was obtained for each animal from the field veterinarians.

Source of environmental materials:

In all, 22 saprobic materials which included 8 soil, 6 water, 4 algae and plants and 4 snails were collected from the vicinity of two positive animals.

Laboratory techniques for clinical materials:

1. Direct microscopy:
### TABLE - 16

Distribution of animals according to sex and age groups

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species of animals investigated</th>
<th>Number of animals examined</th>
<th>Sex</th>
<th>Age groups in years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>1.</td>
<td>Buffalo</td>
<td>41</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>2.</td>
<td>Cattle</td>
<td>39</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>Goat</td>
<td>27</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>4.</td>
<td>Sheep</td>
<td>21</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>5.</td>
<td>Camel</td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>139</td>
<td>58</td>
<td>81</td>
</tr>
</tbody>
</table>
A loopful of nasal exudate was kept on a clean glass slide and treated with 10% potassium hydroxide for 10 minutes and immediately examined under microscope. Smears prepared from nasal secretions were stained with haematxylin and eosin, Periodic acid Schiff and examined microscopically for the presence of cysts and sporangia.

2. Cultural inoculation on laboratory media:
A small piece of polyp and mycopurulent nasal exudate were liberally cultured on to triplicate plates of blood agar, brain-heart infusion agar and Sabourauds dextrose agar with chloramphenicol. Each plate was incubated at 25, 30 and 37°C and examined daily for 4 weeks.

3. Animal inoculation:
About 1 gm biopsied material from nasal mucosa which contain polyps was emulsified in 5 ml sterile physiological saline (0.85%) and centrifused at 3000 r.p.m. for 10 minutes to get saline suspension of sporangia. This suspension was treated with chloramphenicol (0.5 mg/ml) and inoculated by intraperitoneal (0.5 ml) and intranasal (0.2 ml) routes in
two adult male Swiss albino mice (20 gm wt) and two male Guinea pigs (200 gm wt).

Similarly, 1 ml of nasal exudate treated with 1 ml of antibiotic solution of chloramphenicol (0.5 mg/ml) and homogenised with the help of glass beads was administered in laboratory mice and Guinea pig by both the routes. Inoculated animals were kept under observation for 2 months and nasal swabs were examined periodically at an interval of 15 days (15, 30, 45, and 60) for the development of rhinosporidial infection; in any.

4. Sedimentation technique:
In order to rule out the possibility of Schistosoma nasalis, the positive specimens were examined both by floatation and sedimentation technique using saturated sugar solution. The eggs of S. nasalis being heavier settle at the bottom, and mounts were made from the deposit for the presence of this nematode eggs.

Examination of environmental specimens:

1. Cultural technique:
About one gram of soil, snails and algae with plants were homogenised with 5 ml of sterile physiological saline. This suspension was incubated at 37°C for 30 minutes with 2 ml of antibiotic solution containing chloramphenicol (1 mg/ml). Aliquots of 0.1 ml and 0.2 ml were streaked on duplicate plates of blood agar, brain heart infusion agar and Sabouraud's medium with antibiotics. One set of Petri plates were incubated at 37°C and other at 25°C. These were daily examined for the fungus upto a period of 1 months.

The contaminated mud water from different ponds of rural areas was collected in sterilized in McCartney bottle in 10 ml quantity and immediately brought to laboratory for mycological processing. Five milli litre of water was centrifused at 3500 r.p.m. for 15 minutes and the deposit was emulsified with 2 ml of chloramphenicol solution (0.5mg/ml). From this antibiotic treated suspension, 0.1 ml and 0.2 ml were cultured on above mentioned media.
TABLE - 17

Demonstration of *Rhinospordium seeberi* infection in the nasal cavity of diseased animals.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species of animals surveyed</th>
<th>Number of animals investigated</th>
<th>Number of animals positive of <em>R. seeberi</em> infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Buffalo</td>
<td>41</td>
<td>0.0</td>
</tr>
<tr>
<td>2.</td>
<td>Cattle</td>
<td>39</td>
<td>1.0</td>
</tr>
<tr>
<td>3.</td>
<td>Goat</td>
<td>27</td>
<td>1.0</td>
</tr>
<tr>
<td>4.</td>
<td>Sheep</td>
<td>21</td>
<td>0.0</td>
</tr>
<tr>
<td>5.</td>
<td>Camel</td>
<td>11</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>139</td>
<td>2.0 (1.4%)</td>
</tr>
</tbody>
</table>

per cent

6.2 (4.4%)
### TABLE - 18

Clinical details of the two positive cases of nasal rhinosporidiosis

<table>
<thead>
<tr>
<th>Animal species positive</th>
<th>Sex</th>
<th>Age group in years</th>
<th>Clinical signs observed</th>
<th>Duration of illness</th>
<th>Drugs consumed</th>
<th>Coexisting disease, if any</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>1</td>
<td>M 3-5 F 6-8</td>
<td>Persistent copious, muco purulent discharge from both nostrils, polyps about 1.5-2 cm in diameter, present in left nostril, occasion blood tinged discharge from left nostril, stertorous breathing</td>
<td>45 days (Pfizer), Kanacin, Avil, Hoechst</td>
<td>Terramycin, Betnosol (Blaxo)</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>1</td>
<td>M 3-5 F 6-8</td>
<td>Intermittent, unilateral, mucopurulent nasal discharge from right nostril, no blood stained discharge, no polypoid growth in the nostrils, difficulty in respiration</td>
<td>38 days (M&amp;B)</td>
<td>Phenergan, Strepto-Penicillin (Sarabhai), Kanacin (Alembic) Hosta-cortin-H (Hoechst)</td>
<td></td>
</tr>
</tbody>
</table>

---

*Note*:
- M: Male
- F: Female
- M&B: Mineral Bactericidal
- M/B: Mineral Bactericidal
Laterally occurring nasal rhinosporidiosis in a 5-year-old indigenous she-goat which showed intermittent, unilateral mucopurulent discharge from right nostril and dyspnoea.
RESULTS

The occurrence of nasal rhinosporidiosis was studied in 139 ruminants of different breed, age and sex. Of the 139 animals investigated, only 2 (1 cattle, 1 goat) animals showed Rhinosporidium seeberi infection (Table - 17). The positive specimens originated from a 6-year-old male bullock and a 5-year-old she goat (Table - 18). Fig. 14 Rhinosporidiosis could not be detected in buffaloes, sheep and camels. Both the animals had persistent, intermittent, unilateral, bilateral, mucopurulent nasal discharge for the last 38-45 days besides dyspnoea (Table - 18). Occasionally blood stained discharge was noticed from the left nostril of the bullock. The same animal had polypoid growths of about 1.5-2.0 cm in diameter in the left nasal cavity which were latter removed surgically by the Veterinarian. The prolonged treatment with antibiotics (Terramycin - Pfizer, Strepto - Pencillin - Sarabhai, Kanacin - Alembic), cortisone (Betnesol - Glaxo, Hostacortin - H - Pfizer) and antihistamine (Phenergan - May and Baker, Avil - Hoechst) proved futile. History of trauma to the nasal mucosa
### TABLE - 19

Distribution of positive nasal rhinosporidiosis animals according to the source water supply and traumatic injury to the nostril

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Animal</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Distribution of positive cases according to:</th>
<th>Source of water supply</th>
<th>Condition of the nasal mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tap water well</td>
<td>Stream Pond</td>
</tr>
<tr>
<td>1.</td>
<td>Buffalo</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Cattle</td>
<td>39</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Goat</td>
<td>27</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>Sheep</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>Camel</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>139</td>
<td>2</td>
</tr>
</tbody>
</table>

---

Source of water supply: Tap water, Stream, Pond
Condition of nasal mucosa: Healthy, Injured
A mature sporangium in the stroma of a polypoid lesion collected from a 6-year-old male cattle showing few endospores. KOH Mount X 200.
FIGURE 16

Thick-walled sporangia (cyst) of *Rhinocerosporidium seeberi* in a smear of nasal exudate from a 5-year-old she-goat. Haematoxylin and Eosin X 200.
varying degree of vacuolation and degeneration.

In addition, homogeneous eosinophilic material
by mononuclear cells and a few epithelial cells
and eosin stained smears; sporulation were surrounded
prominent and at the presence of 5 - um endospores.

In the greater, In case of few mature sporangia, the wall

um in diameter introducing different stage of develop-

most (20 - 70) sporangia measured from 1 to 276

round, then watered cysts (sporangia) of various

The wet mounts and for preparations showed

In the greatest section of diseased animals
as no one of the stromailes resists it could be demonstrated
eggs and cysts at so case uniformly resistant
sedimentation technique employed for the parasites

negative for other fungi and bacteria. The

In addition, the specimens were also

culture media nor in the experimental laboratory

neither be tested on the various environmental

The etiological agent, Pythium interrogatum, isolated could

from pools and ditches of pastures (Table - 19).

both the animals were drinking contaminated water

animals had any symptoms of underlying disease.

was attributable only in the cattle. None of the
Investigation of saprobic material and clinical specimens for the recovery of *Rhinosporidium seeberi* in experimental animals and in different laboratory media.

<table>
<thead>
<tr>
<th>Type of specimen examined</th>
<th>No. examined</th>
<th>Number of laboratory animals yielded fungus and denominator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucopurulent nasal discharge</td>
<td>9/0</td>
<td>9/0</td>
</tr>
<tr>
<td>Polyps from biopsied snails</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>Plants</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>Pond Water</td>
<td>12/0</td>
<td>12/0</td>
</tr>
<tr>
<td>Soil</td>
<td>16/0</td>
<td>16/0</td>
</tr>
</tbody>
</table>

For each type of specimen, the table shows the number of plates positive for fungus and the number of Petri dishes inoculated with the specimen. The table also lists the type of laboratory media used, such as Blood-agar, Brain-Heart Infusion medium, and Sabouraud's agar. This information is critical for the research on the recovery of *Rhinosporidium seeberi*.
spores were also detected. Sporangia with thick, cellulose-like walls could be easily demonstrated in the nasal exudate of a 5-year-old she goat by haematoxylin and eosin method (Fig. 16).

Attempts of culture *Rhinocystis seeberi* from the positive clinical material both in laboratory media and experimental laboratory animals were unsuccessful. Further, the fungus could not be demonstrated in any of the 22 environmental samples obtained from the vicinity of the positive animals (Table 20).
DISCUSSION

The present study reveals 1.4 per cent prevalence of nasal rhinosporidiosis in domesticated ruminants. The diagnosis in both the cases was confirmed by the demonstration of sporangia and sporangiospores in wet mounts, KOH preparations and haematoxylin and eosin stained smears. It was interesting to note that the bullock was constantly passing copious, mucopurulent nasal discharges from both the nostrils. However, most of the earlier workers have reported unilateral involvement of the nasal mucosa in this disease (Ainsworth and Austwick, 1973; Blood et al., 1981). This emphasizes the further investigations to study the clinical spectrum of rhinosporidiosis which is endemic in Indian as well as Argentina.

Although the exact mode of infection and pathogenesis of rhinosporidiosis is not well understood, it has been postulated that traumatic injury to the mucosa may act as an important predisposing factor in the initiation of the infection (Karunaratne, 1964; Maddy, 1967; Smith et al., 1972). The same is true in the present case as the cattle received trauma by a sharp wooden splinter on the left
nostril while ploughing the agricultural land. It is however, safe to assume that accidental injury to nasal mucosa by possible contaminated soil, dust laden splinter may perhaps be the mode of infection. A similar finding has been reported by Jimenez and co-workers (1984) in ocular rhinosporidiosis of human beings. Further, it has been reported that man and animals may become infected by splashing the contaminated water into the nostrils while drinking (Maddy, 1967). In one study Mandlik (1937) recorded 20 per cent infection rate in a group of workers engaged in removing sand from a river bottom. This view was latter substantiated by Nino and Freire (1964) that organism may be present in water and thus invade the nasal mucosa of man and animals. Likewise Paul, Khan and Gupta (1981) also mentioned that 13 out of 19 patients who had bathed in a pond which was frequented by the animals acquired the infection. It is pertinent to mention here that in man the disease is commonly associated with people living in crowded, unsanitary conditions or in those who swim in stagnant water or water commonly used by livestock (Kutnick and Kerth, 1976).

Zsckokke (1913) is credited for the first report of animal rhinosporidiosis by describing the disease
in a horse. Since then infection has been recorded in many species of animals with frequency in equines (Ayyar, 1932; Smith and Frankson, 1963; Ainsworth and Austwick, 1973; Londero, Santos and Freitas, 1977; Payan and Buendia, 1980). The disease is cosmopolitan in distribution, however, the prevalence rate in human beings is higher in India particularly in the States of Kerala and Tamil Nadu (Chitravel et al., 1980). Further, the infection rate was reported to be higher in males (Karunaratne, 1964; Minó and Freire, 1964).

In this context Rao (1933) mentioned the association of rhinosporidiosis with nasal schistosomiasis. He also recorded that disease is more frequently encountered in bullocks than cows. The plausible explanation of this was that the nasal septum of young bullocks at the age of three and a half year is usually punctured for nose string and then the bullocks are used for ploughing the land, on the contrary, the cows are not utilized for agricultural work.

Grover (1970) has claimed that he could succeed in demonstrating the maturation of sporangia and spores in TC 199 synthetic liquid. However, our preliminary work was unable to isolate the etiological agent from biopsied material and nasal exudate both
in enriched media and laboratory animals. This observation is in agreement with the earlier work of de Mello (1949) who could neither grow the organism on cultural media nor produce experimental transmission of the infection in man and animals. Failure to culture *R. seeberi* in vitro left us a challenging area for future research which may enhance our understanding on the life history of this complicated microorganism.

Epidemiological investigation conducted to find out the source of infection in saprobic environment and also to study the life history of the disease failed to yield any encouraging results. This finding is in consistent with Reddy and Lakshmi (1962) who failed to recover the fungus from various environmental materials.

It has been emphasized that *Rhinocporidium seeberi* infection should be considered as one of the important etiological agent of chronic rhinitis in animals particularly those negative for nasal aspergillosis, penicillois, schistosomiasis and nasal granuloma due to *Helminthosporium* spp.
SUMMARY

The present study was undertaken in 139 domesticated ruminants to find out the prevalence of nasal rhinosporidiosis. In all 139 nasal secretions originated from 41 buffaloes, 39 cattle, 27 goats, 21 sheep and 11 camels were examined for *Rhinosporidium seeberi* infection. Rhinosporidiosis could be diagnosed in two animals giving a prevalence of 1.4 per cent only. The positive specimens originated from a 6-year-old male indigenous cattle and a 5-year-old she goat. The disease could not be detected in buffaloes, sheep and camel.

The diseased animals exhibited intermittent, persistent, unilateral, bilateral, mucopurulent nasal discharge and dyspnea for the last 33-45 days. However, polyps and blood stained discharge could be observed in the left nostril of a bullock. There was no history of any underlying disease such as nasal schistosomiasis, nasal granuloma, allergic rhinitis, bacterial rhinitis, *Aspergillus* rhinitis etc. However, nasal mucosa of bullock was injured by a sharp wooden splinter. Both the animals gave a history of extensive and indiscriminate use of antibiotics, corticosteroid and antihistamines without any clinical response.
Diagnosis was confirmed by the clinical history, demonstration of thick walled sporangia and sporangiospores in the nasal exudates of the diseased animals by KOH mounts and haematoxylin eosin stained smears. No bacteria or fungi could be isolated from the clinical material on blood agar, brain heart infusion agar and Sabouraud's medium. The specimens were also negative for the ova of Schistosoma nasalis when examined by floatation and sedimentation techniques.

*Rhinosporidium seeberi* could neither be isolated on the various enrichment media nor in the experimental animals, and therefore, leaves an challenging area for future research.

The epidemiological findings failed to establish the source of infection as the fungus could not be recovered from 22 environmental samples collected from the immediate surrounding of the diseased animals.

Since nasal rhinosporidiosis may simulate clinically with nasal schistosomiasis, aspergillosis and nasal granuloma, it should be differentiated from these conditions by laboratory tests.
REFERENCES


Fain, A. and Herm, V. 1957. Two cases of nasal rhinosporidiosis in a wild geese and a wild duck. Mycopathologia 8: 54-61.


