

PATTERNS OF RHINOSPORIDIOSIS IN SRI LANKA: COMPARISON WITH INTERNATIONAL DATA

SN Arseculeratne, S Sumathipala and NB Eriyagama

Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka

Abstract. One hundred forty-three cases of rhinosporidiosis, confirmed by smear or biopsy, treated in two major General Hospitals in Sri Lanka over a 14 year period (1995 - 2009) were analyzed in regard to their epidemiological, clinical, clinico-pathological, immunological and microbiological features. Regional variations in incidence, age and sex distribution, bathing history, and histopathology were seen. Lacustrine waters were the commonest probable source of infection (84%). Rivers were a source of *Rhinosporidium seeberi* in Sri Lanka (11%) and domestic well water was a probable source in 5%. The epidemiological features, clinical presentations and histopathology were similar to those in other series. The antirhinosporidial antibody (mean) titers were IgM - 142.1 and IgG - 178.5, compatible with rhinosporidiosis of long duration. Mantoux positivity to PPD was found in 65% of normal Sri Lankans, by only 35% of patients with rhinosporidiosis. No outbreaks have been reported in Sri Lanka or India. No animal cases of rhinosporidiosis have been reported in Sri Lanka, although rhinosporidiosis in animals has been repeatedly documented in India.

Key words: human rhinosporidiosis, *R. seeberi*, epidemiology, clinico-pathology, immunological features, Sri Lanka

INTRODUCTION

Rhinosporidiosis has been reported from about 70 countries world-wide including Europe, North and South America and the sub-Saharan and wet regions of Africa. Sri Lanka and India are hyperendemic for rhinosporidiosis. Other countries with high endemicity are Brazil, Uganda and the southwestern USA.

Occurrence of rhinosporidiosis in countries with no previous cases is now being reported, mainly in persons from endemic regions in those seeking employ-

ment. Rhinosporidiosis in animals from endemic areas has also been described (Leeming *et al*, 2007). This disease may be an emerging infectious disease.

Only one comprehensive review of Sri Lankan cases of rhinosporidiosis exists (Karunaratne, 1964). The present series is a second report that comprises a further 143 cases of rhinosporidiosis in humans seen from 1995 to 2009, in the General Hospital, Kandy, Central Province (Fig 1), and in the General Hospital, Anuradhapura, a rhinosporidiosis-endemic area in North Central Province, with referrals from peripheral areas in the country. These two major hospitals and the smaller peripheral hospitals that we received specimens from, would have received the large majority of patients

Correspondence: SN Arseculeratne, Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka.
E-mail chubby@slt.net.lk

with rhinosporidiosis and the cases could therefore be taken as representative of the Sri Lankan situation in regard to rhinosporidiosis since no information or specimens were sent from other regions of the country.

Our data are compared with Karunaratne's data (1964) of Sri Lankan cases up to 1964, which is the only major review of Sri Lankan cases apart from the present report, and with Indian publications (Allen and Dave, 1936; Satyanarayana, 1960; Jain, 1967; Kameswaran, 1986; Billore, 1995; Sudharshan *et al*, 2007); a report of 17 patients from Serbia (Vucovic *et al*, 1995), while reports of sporadic cases from other countries exist. Some immunological features of the Sri Lankan patients were reported earlier (de Silva *et al*, 2001; Arseculeratne *et al*, 2004). The reports from other countries, with the exception of one report from India (Chitravel *et al*, 1981), did not include immunological findings.

MATERIALS AND METHODS

Patients

Hospital case records and interviews with patients were used to gather data. Conventional laboratory procedures were used for blood and Mantoux testing. Tests of antirhinosporidial cell-mediated and humoral immune status were carried out as described by de Silva *et al* (2001) and Arseculeratne *et al* (2004). According to routine ward practice, consent for documentation of case records and operation were obtained from the patients.

Cell mediated immunity

Cell-mediated immunity (CMI) was examined in 7 patients by (a) immuno-histochemistry with labelled antibodies to specific markers on immunologically competent cells; (b) lymphoproliferative responses of peripheral blood lymphocytes

in 14 patients, and in asymptomatic persons, *in vitro*, to non-specific mitogen Phytohemagglutinin-PHA and to rhinosporidial antigen; (c) responses in 40 patients to the Mantoux Purified Protein Derivative (PPD) test for *Mycobacterium tuberculosis* as an indicator of immunological (CMI) competence.

Humoral immune status.

Anti-rhinosporidial antibody titers in patients with rhinosporidiosis, were assayed by immuno DOT-blot tests against rhinosporidial antigens released from suspensions of endospores and juvenile sporangia by ultrasonic disintegration.

RESULTS

Rhinosporidiosis

One hundred forty-three cases admitted directly to the General Hospital, Kandy, or referred from the General Hospital, Anurdahapura an endemic area for rhinosporidiosis, or referred from other peripheral hospitals, mainly Kurunegala or Trincomalee, also rhinosporidiosis-endemic areas (Fig 1), were studied.

Geographical distribution

The geographical distribution of 142 cases whose locality of residence were known, is shown in Fig 1. The highest prevalence was in the Dry Zone, while Kandy in the Wet Zone had 8% of all cases; this distribution is discussed below. The regions that had a lower prevalence (≤ 2 cases) are classifiable into their climatic zones as follows: Dry Zone ($n=30$, 59%); Intermediate Zone ($n=7$, 14%); Wet Zone ($n=14$, 27%).

The greater prevalence of cases in the Dry Zone, than the Intermediate and Wet Zones, is noteworthy.

Probable sources of infection

In 95 cases in which the bathing his-



Fig 1—The regional distribution of 137 cases of rhinosporidiosis in humans in Sri Lanka. The Dry and Intermediate Zones are separated by a bold line; the Wet and Intermediate Zones are separated by an interrupted line. Dots represent 15,000 village tanks. Figures represent percentages of total number of cases: 137.

tory of the patients was available, the overall percentage distribution of exposure to ground waters, and their relation to site of rhinosporidiosis is shown in Table 1. These figures indicate the predominant water source to which the patients were exposed to was lacustrine water in reservoirs.

Duration of disease

Nasopharyngeal cases had the longest history of disease, ranging from 3 to 30 years, with a mean of 10 years; ocular rhinosporidiosis had a shorter history of less than 8 years. In both upper respiratory and ocular rhinosporidiosis the majority of cases had a history of less than one year. The 5 disseminated cases had a history of over 8 years (Table 2): In 99 cases, a majority (57%) had a range in years from 1 to 55, followed by 39% with a range from 1 to 9 months, and 4% with ocular rhinosporidiosis had a range from 2 to 3 weeks.

Sites

Table 3 records the distribution by site of infection in the 143 cases. The inferior turbinate and septum were the commonest nasal sites.

Recurrences

The overall total rate of recurrence in the 137 cases was 37%; the highest rate was in disseminated rhinosporidiosis (100%)

Table 1

The bathing history of 95 patients; the numbers refer to the percentage distribution of patients that bathed in reservoirs or lakes (total 83), in rivers (total 12), and garden wells (5).

	Percentage distribution		
	Upper respiratory tract	Ocular	Disseminated
Reservoirs and lakes	88	67	75
Rivers	8	28	0
Garden wells	4	5	25

Table 2
Duration of rhinosporidiosis in relation to site.

Duration (years)	Number of cases (%) total 95				
	< 1	1-3	4-7	8-10	>10
Upper respiratory	29 (31)	15 (16)	7 (7)	12 (13)	12 (13)
Ocular	13 (15)	1 (1)	1 (1)	-	-
Disseminated	-	-	-	2 (2)	3 (3)

Table 3
The sites in 143 cases of rhinosporidiosis.

Site	Number of cases (%)
Upper respiratory tract	^a 112 (78)
Nasal	82 (57)
Nasopharyngeal	30 (21)
Ocular	25 (18)
Scleral	18 (13)
Palpebral	7 (5)
Urethral / penile	1 (0.7)
Disseminated	5 (3.5)

^a One patient had nasal and ocular rhinosporidiosis and 1 had nasal rhinosporidiosis with dacryocystitis of unconfirmed etiology.

followed by rhinosporidiosis of the upper respiratory tract (38%), and the lowest with ocular rhinosporidiosis (15%).

Histopathology of rhinosporidial tissues

A uniformity in the histopathological reactions was seen in 64 cases from diverse clinical presentations, nasal and nasopharyngeal, ocular, disseminated, and one urethral case. This uniformity was in respect to the host's stromal reactions (edema, hemorrhage, cystic spaces, fibrosis) and cell infiltration patterns (mononuclear cells – lymphocytes and macrophages, neutrophils, and very rarely Langhans giant cells). The Splendoré-Hoepli reaction, commonly seen in classical mycoses, was absent, and granuloma formation was uncommon.

Unusual histopathology (Arseculeratne *et al*, 2001) included variations in the density of the cell infiltrate from minimal to heavy especially around sporangia, and abnormalities of the pathogen – absence of sporangial walls, degenerate and thick sporangial walls. The histopathology did not correlate with the clinical pattern or with immunological features, nor did the degree and composition of the cell infiltrate correlate with the intensity of rhinosporidiosis.

All ontogenic stages of *R. seeberi*, endospores, trophocytes, sporangia [juvenile, intermediate (immature) and mature] were present amidst cell infiltrates.

The patients

Age. One hundred thirty-six patients had a decreasing order of incidence in the following age groups: 21-30, 31-40, 11-20, 41-50, 51-60, 0-10, 61-70 and over 70. Sixty percent of the cases were in the age group 11-40; the maximum incidence of 26% was in the age group 21-30.

Sex. The distribution of 137 cases according to sex of the patient is shown in Table 4. The male : female ratio was 2.3:1 in respiratory sites, 2:1 in ocular sites, and 4:1 in disseminated cases.

Distribution. In this series 4.9% were Muslims, 4.2% were Tamils and 91% were Sinhalese. Ocular rhinosporidiosis was not recorded in either Muslims or Tamils.

Table 4
The percentage sex distribution in 137 cases of rhinosporidiosis.

	Upper respiratory sites	Ocular sites	Disseminated
Total number/site	111	21	5
Male	70%	67%	80%
Female	30%	33%	20%

Bathing history. In 95 cases from whom a bathing history was obtained, 83% had regularly bathed in reservoirs, 12% had bathed in rivers and 5% in garden wells. Thus, lacustrine waters (reservoirs and rivers) were the commonest probable source of infection. The data from Kandy in the Wet Zone (Fig 1) shows that of 11 cases, 5 (45%) were ocular. This is of significance since the country's largest river and its tributaries pass through this district. Ocular cases in Anuradhapura in the Dry Zone (Fig 1) that has stagnant lacustrine waters, comprised only 14% of the total number of cases.

There was no major differences between sexes in exposure to these two sources: 89% of males and 79% females had exposure to reservoirs, while 11% of males and 21% of females had exposure to rivers.

Anti-rhinosporidial immunity status.

(a) Non-specific immunity. Eight samples from 7 patients were examined. Of the cells with non-specific immunity, CD68+ macrophages were uniformly present. Neutrophils were marked around free endospores. Giant cells were rare.

(b) Cell mediated Immunity (de Silva *et al*, 2001)

Immuno-histochemistry. In 7 patients CD3+ and CD4+ helper lymphocytes were scarce while CD8+ T-lymphocytes were numerous, especially around the en-

dospores, and within mature sporangia. There were many TIA-1 positive lymphocytes of the cytotoxic subtype. CD56/57 natural killer lymphocytes were present around sporangia.

Lympho-proliferative responses (LPR). Of 14 patients, 9 showed depressed (<1, anergy) stimulation indices (SI) in response to rhinosporidial antigen, although they did not show significant differences from the LPR to the non-specific mitogen Con A, indicating that specific immunosuppression (to rhinosporidial antigen) was present in the patients. There was no correlation between the SI (whether < 1 or > 1) and the pattern of clinical disease, the site, duration, number of lesions, or whether the disease was localized or disseminated.

Mantoux reactivity

Table 5 records the Mantoux reactivity to PPD (*Mycobacterium tuberculosis*) in 40 patients. In 40 patients, 26 (65%) with disease in all three sites were Mantoux-negative. In the 26 Mantoux-negative patients, 21/26 (81%) had nasal/nasopharyngeal rhinosporidiosis and 12% had ocular disease. Both disseminated cases were Mantoux-negative.

Humoral immunity (Arseculeratne *et al*, 2004). CD20 + B cells and plasma cells were present in considerable numbers in the rhinosporidial tissues in the 7 patients examined. Anti-rhinosporidial antibody

Table 5
The distribution of Mantoux reactivity in 40 patients with rhinosporidiosis.

	Number of patients		
	Nasal-nasopharyngeal	Ocular	Disseminated
Mantoux positive 35%	12	2	0
Mantoux negative 65%	21	3	2

Table 6
The major blood group distribution in 51 patients with rhinosporidiosis and in Sri Lanka's population.

Blood group	Percentage of cases	National data (%)
A	24	21
B	24	26
O	45	43
AB	7	5

(geometric mean) titers in 34 patients with rhinosporidiosis, were IgM-142.1 and IgG-178.5, while titers in 29 normal asymptomatic persons living in the same geographical area were IgM-169.6 and IgG-62.8.

Blood groups. In 51 cases in which the blood group was known, the distribution according to group is shown in Table 6, in comparison with national figures in Sri Lanka's general population: the relative incidence of rhinosporidiosis in the 4 blood groups paralleled the blood group distribution in the general population.

The distribution, (percentage within brackets) of the site of rhinosporidiosis according to the blood group in 51 cases is shown in Table 7; the relative distribution of blood groups in rhinosporidiosis in different sites also paralleled that in the general population.

The Pathogen - *Rhinosporidium seeberi*
Genetic heterogeneity in strains of *R. seeberi*. Six strains of *R. seeberi* in 6 cases of this series, one unilateral nasal, one bilateral nasal, one nasopharyngeal, one oropharyngeal and two disseminated cases of rhinosporidiosis, were found by the Random Amplification of Polymorphic DNA (RAPD) test with the random BOX primer and UPGAMA Dice Coefficient analysis, to fall into 3 groups A, B and C, with 2 in each (Fig 3), with amplified rhinosporidial bands ranging from 200 to 1,600 bp. The control bands from nonrhinosporidial human tissues had no bands corresponding to the bands referable to *R. seeberi* from rhinosporidial tissues. RAPD Group A had 2 strains, from bilateral nasal and disseminated rhinosporidiosis; Group B had 2 strains from oropharyngeal and disseminated rhinosporidiosis and Group C had 2 strains from nasopharyngeal and unilateral nasal rhinosporidiosis, respectively.

There was no correlation between RAPD group or strains within each group and the clinical pattern of disease.

Sensitivity to biocides and antimicrobial drugs

Sensitivity to biocides. Endospores from two cases, tested individually, were exposed to biocides *in vitro* and assayed by the MTT-reduction test for viability

Table 7
The distribution of blood group in relation to site of rhinosporidiosis.

	Blood Group			
	A 24 %	B 24 %	O 42 %	AB 10 %
Number of cases				
Respiratory	9	12	17	4
Nasal	7	7	12	3
Naso-pharyngeal	2	5	5	1
Ocular	3	0	3	1
Scleral	3	0	2	1
Palpebral	0	0	1	0
Disseminated	0	0	1	0
Blood group, total number	12	12	21	5

All cases except one were Rh +.

Table 8
Comparison of Indian and Sri Lankan data (Karunaratne, 1964) on percentage, site distribution and sex of patients.

Site	Male		Female	
	India	Sri Lanka	India	Sri Lanka
Nose	74	65	68	57
Eye	5	27	7	35
All other sites	21	9	26	8

(Arseculeratne and Atapattu, 2004). Hydrogen peroxide, glutaraldehyde, chloroxylenol, chlorhexidine, cetrime, thiomersal, 70% ethanol, iodine in 70% ethanol, 10% formalin, povidone-iodine, sodium azide and silver nitrate, in concentrations that are used clinically or in the laboratory, were found to cause total inactivation (Arseculeratne *et al*, 2006). The following brand named biocides, Bacillo-floor, Sokrena and Sterillium, were also effective and caused severe morphological damage to walls and contents of the endospores while Bactolin, Bodedex, Cutasept and Korsolex produced less intense damage (Eriyagama and Arseculeratne, 2007).

Sensitivity to antimicrobial drugs. Using the MTT-reduction test (Arseculeratne and Atapattu, 2004) as an indicator of rhinosporidial endospore viability, the following drugs were found to have the respective mean inhibitory concentrations ($\mu\text{g/ml}$) at the 50% end-point (IC_{50}) on 4 different strains of *R. seeberi* from patients with different clinical presentations: amphotericin B (57.1), dapson (29.7), ketoconazole (51), trimethoprim-sulphadiazine (38.4) and sodium stibogluconate (55.7); regarding drugs for veterinary use, Berenil (12.5) and Imizol (9) (Arseculeratne *et al*, 2008). Recent data indicates that cycloserine with a mean IC_{50} of approximately

Table 9
The percentage ethnic distribution of nasal and ocular rhinosporidiosis in Sri Lankan males (Karunaratne 1964).

Distribution	% in population	Site of rhinosporidiosis	
		Nasal	Ocular
Sinhalese	67.4	46	67
Ceylon Tamils	11	17	12
Indian Tamils	13	12	4
Muslims	6.7	22	16

10 µg/ml, echinocandin (caspofungin) (IC₅₀ 2.7 -1 trial, <0.02 µg/ml - 5 trials) and Voriconazole (IC₅₀ 0.03 µg/ml - 4 trials) (Arseculeratne, 2009; unpublished data) could be supplementary chemotherapeutic agents for rhinosporidiosis.

DISCUSSION

Karunaratne's data (Karunaratne, 1964) for Sri Lanka is not strictly comparable with the present series because he, in Colombo in the Wet Zone on the western coast, received "very few specimens from outstation hospitals", while the cases in the present series were from areas that were excluded from Karunaratne's series (Karunaratne, 1964) but were from the Dry and Intermediate Zones that are rhinosporidiosis-endemic regions of the country.

The majority of patients with rhinosporidiosis in all sites lived in the Dry Zone in Sri Lanka (Fig 1). Apart from the District of Jaffna in the Northern Province (from which patients and data were difficult to access), the geographical contiguity of the Dry Zone of Sri Lanka and the southern regions of India, separated only by the Palk Strait (Fig 2) might have a correlation between the relatively higher incidence of the disease in southern India (Tamil Nadu) and the Dry Zone of Sri

Lanka. The southern regions of Tamil Nadu (below Madurai, from where many cases have been reported) and the Dry Zone of Sri Lanka are geographically similar (Farmer, 1956); the Wet Zone of Sri Lanka has geographical similarities with Kerala.

Indian data summarized by Kameswaran (1986) is presented in map form (Fig 2) to emphasise the geographical proximity of India to Sri Lanka.

Both India and Sri Lanka show regional variations in the prevalence of rhinosporidiosis while the geographical features in the high and low incidence regions of the two countries are broadly similar. According to the data recorded by Kameswaran (1986) the highest incidence in India is in Tamil Nadu followed by Kerala. In Sri Lanka the townships with the highest incidence are in the Dry Zone (Fig 1) which is geographically comparable with the eastern regions in Tamil Nadu, and in Kandy in the Wet Zone which is comparable with Kerala, in India. Data regarding the presence of lacustrine collections of ground water in Tamil Nadu is not available to us, although in Sri Lanka, the Dry Zone is characterized by the presence of 15,000 major and minor "tanks" of lacustrine waters (Fig 1).



Fig 2—The regional distribution of rhinosporidiosis in India. The figures refer to the average number of cases per year. (Adapted from the data of Kameswaran, 1986).

Variations between states in India (Billore, 1995) are comparable with variations seen between districts in Sri Lanka, eg, Anuradhapura (Fig 1) had more patients (35) than Polonnaruwa (6) in the Dry Zone. The States of India showed major differences in incidence (Fig 2), the districts of Anuradhapura (Dry Zone) and Colombo (Wet Zone) showed in Sri Lanka. Tamil Nadu had the maximum of 1,989 cases followed by Madhya Pradesh with 772, while Karnataka had 7 (Billore, 1995). Reports from other countries do not describe regional variations except in the south-central US.

A decreasing incidence of rhinosporidiosis is now seen in Sri Lanka, an experience found by Indian researchers (AJob,

2009, personal communication).

As in Karunaratne's Sri Lankan cases up to 1964, there were no cases in our series that indicated human to human transmission. Karunaratne (1964) cited four Indian reports that had the same conclusion (Cherian and Vaseudevan, 1929; Kurup, 1931; Cherian and Satyanarayana, 1949; Satyanarayana, 1960); Billore (1995) in India concluded likewise. This exclusiveness and the absence of patient-to-person transmission probably indicate susceptibility factors in the individual host and secondary rhinosporidial growth have occurred in the same patient (Allen and Dave, 1936) on opposing surfaces, such as in the urethra and nose.

The incubation period that could not be assessed because the date of first exposure to *R. seeberi* in the present series could not be specified, nor could the first appearance of the lesion be dated in view of its insidious onset and slow growth, and chronicity, except in the eye. In one case of bulbar rhinosporidiosis, predisposing trauma to the eye and the development of the disease were separated by 13 years. Seasonal incidence is not discernible because of the slow manifestation of the disease and because the time of arrival of the patient in the hospital is influenced by the exigencies of profession, and transport from rural areas and while the disease seldom poses an acute emergency.

Karunaratne (1964) recorded nasal disease predominated at 63% over ocular disease (27%), while Billore (1995) in India noted that 83% were nasal and 8% were ocular. Karunaratne's data (1964) for Sri Lanka gave the following percentage distribution in a total of 657 cases, by site and sex of patient, indicating a greater occurrence of nasal disease in males (65% compared with 57% in females) and a greater

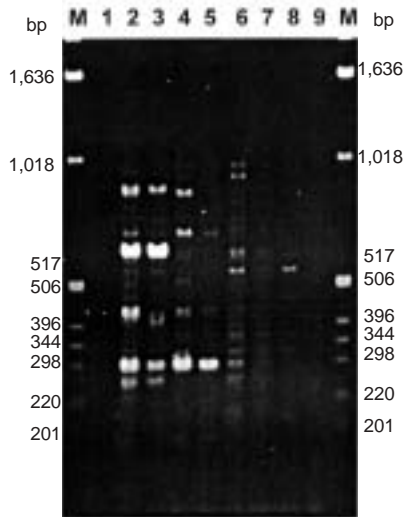


Fig 3—The RAPD pattern in 6 strains of *R. seeberi* from six human cases of rhinosporidiosis. Finger prints obtained by BOX-PCR; Lanes M: 1-kb DNA ladder; lane 1: *R. seeberi*-containing human tissue (positive control). Lanes 2 – 7: *R. seeberi* from 6 different patients. Lane 8: human leukocytes and lane 9: human liver/spleen cells (negative controls).

occurrence of ocular disease in females, (35% compared with 27% in males).

Our data agrees with the other reports that the upper respiratory tract was the commonest sites of rhinosporidiosis, followed by the eye. Sudharshan *et al* (2007) reported that in 462 cases in India, the largest single series, 81% were in nasal or nasopharyngeal sites, 14% were in ocular sites and 7% were with disseminated rhinosporidiosis. Our findings on the site of nasal disease also agree with those of Karunaratne (1964) in Sri Lanka, in India and in other countries, that the commonest sites in nasal disease are the septum followed by the inferior turbinate.

Whether the flowing river water in Kandy (where 45% of cases were ocular), with its suspended sand particles was

more likely to have abraded the ocular mucosa as a predisposition to ocular rhinosporidiosis, than stagnant waters is worthy of consideration. Noronha's cases (1933) of river sand-workers were largely nasal but included a few ocular cases, while Mandlik's patients (1937) all had nasal rhinosporidiosis. In our series, there was only one nasal case that was occupied in sand-retrieving while Noronha's and Mandlik's patients were all sand-workers who dived in to retrieve sand.

Karunaratne's data for Sri Lanka (1964) indicated a shorter duration of ocular than nasal rhinosporidiosis, while it was shorter in females in both sites. In our series of 99 cases, the majority (57%) had a range of duration from 1 to 55 years, followed by 39% with a range from 1 to 9 months, and 4% had a range of 2 to 3 weeks with ocular disease having a shorter duration, probably due to earlier recognition.

Karunaratne's data for Sri Lanka (1964) showed that in nasal rhinosporidiosis, males had a 28% recurrence rate in 53 cases while females had a recurrence rate of 60%. In ocular rhinosporidiosis, males and females had 100% recurrence in 10, and 5 cases, respectively. Billore (1995) recorded a recurrence rate of 21% in 49 cases with nasal rhinosporidiosis, similar to Sri Lanka.

The closest similarities between the cases in Sri Lanka, India and in other countries lay in the histopathology, including (a) epithelial changes, (b) stromal reactions, (c) cell-infiltration patterns, (d) and the ontogenic stages of the pathogen.

(a) Billore (1995) described invaginations of the epithelium, an appearance also described as an early stage in the phenomenon of Trans-Epithelial Elimination. These invaginations enclosed sporangia

which were also found sub-epithelially with the epithelium attenuated leading to its rupture and liberation of endospores. Billore (1995) commented "the epithelium is so greatly thinned"; Billore quoted Nanda *et al* (1996) on intra-epithelial sporangia. Karunaratne (1964) described this appearance in Sri Lankan cases and attributed it to "trans-epithelial infection".

(b) The Indian cases had similar stromal reactions of fibrosis, myxoedematous change, vascularity, cystic spaces, and hemorrhage.

(c) The composition of the stromal cell-infiltrate was similar with giant cells within the sporangia. The Indian reports do not mention the Splendoré-Hoepli phenomenon, as in the Sri Lankan cases. The Indian reports did not include immuno-histological characterization of the cell infiltrate.

(d) All the ontogenic stages of the pathogen were present in the Sri Lankan and Indian cases.

The appearances in (a), (b), (c), and (d) were universal findings.

The absence of a correlation between the nature and progress of rhinosporidiosis and the intensity and composition of the inflammatory cell infiltrate is in contrast to that in malignant tissues where the nature and intensity of the cell infiltrate affords a prognosis on the progress of the malignancy.

Billore's review (1995) of 18 studies with a total of 2,368 Indian patients recorded the maximum incidence, as in the Sri Lankan series, in the 11-40 age group, with 71% of the total number of cases, compared with Sri Lanka's 60%. The age groups 41-50 had the same incidence-status in both series while the differences between the two series lay in the Sri Lankan

5th position of decreasing incidence having been occupied by the 51-60 age group in Sri Lanka while in India the 5th in order of decreasing incidence having been in the 0-10 age group.

As in Sri Lanka, Billore (1995) reviewing 17 reports of a total of 2,336 cases in India, found the highest incidence in the 21-30 age group. In the largest single report of 462 Indian cases, Sudharshan *et al* (2007) reported the highest incidence was in the 21-30 age group. In both series, cases occurred at ages over 70 with the two Sri Lankan cases being ages 70 and 75; in the latter case the duration was 9 months. If the history in the latter case is correct it indicates the disease can be acquired even at an age as late as 75 years. The age range in India was from 4 to 90 years while in our series, the age range was 5 to 70 years; Karunaratne's series of Sri Lankan patients up to 1964 gave an age range from 4 months to 94 years. The only large series of patients (17) in Europe had the majority in the second decade (Vucovic *et al*, 1995).

No clear conclusions could be made regarding the sources from which the pathogen in Sri Lanka was acquired, in relation to the age of the patient.

Karunaratne (1964) noting the comparative rarity of rhinosporidiosis in Indian females, compared the approximate percentage sex distribution according to site in Sri Lanka and India, as shown in Table 8; nasal rhinosporidiosis showed the highest incidence in both sexes in Sri Lanka and India while in ocular rhinosporidiosis, the percentage incidence in Sri Lankan males and females was appreciably higher than in India.

The Indian data summarized from Billore (1995) whose compilation of 21 reports by other workers on a total of 2,450

cases (1,780 males, 670 females) with rhinosporidiosis of the upper respiratory tract and ocular sites in India, found the ratio of males to females to be 2.7:1 in respiratory sites, and 2.5:1 in ocular sites.

The Sri Lankan data given above show similarities with the Indian data, with a male : female ratio of 2.3:1 in respiratory sites, 2:1 in ocular sites, and 4:1 in disseminated cases, with an overall predominance in males, as seen with Karunaratne's (1964) data. Vucovic *et al* (1995) from Serbia reported the male : female ratio was 1.4:1 for both ocular (12) and nasal (5) cases.

In the Sri Lankan patients of this series there were no major differences in exposure to the probable source of infection (reservoirs and rivers) between sexes despite a sex difference in occurrence of rhinosporidiosis.

Our data provides confirmation rhinosporidiosis occurs predominantly in the males, as found in the majority of international reports. This difference is apparently not related to occupation nor to exposure to the natural habitat of *R. seeberi* via lacustrine waters, in which both males and females, especially in rural areas from which our cases were derived, regularly bathe. The predominant occurrence of the disease in male animals also excludes occupational exposure but emphasizes exposure to the natural habitat of *R. seeberi*. The male preponderance is probably an inherent feature of the gender and not attributable to occupation or bathing habits because Wright (1922) in India reported that no girls exposed to the same environment and swimming pool as the infected boys, developed ocular rhinosporidiosis.

The distribution may be related to personal or occupational habits in regard to the occurrence of the disease and the site of the disease; *eg*, penile rhinosporidi-

osis is claimed to be commoner in Muslims, while Karunaratne (1964) regarded paddy cultivation as a predisposing factor noting that both Sinhalese and local Tamils engage in paddy cultivation.

Karunaratne (1964) compared the incidence of nasal and ocular rhinosporidiosis in Sri Lankan males, with the population distribution of races in Sri Lanka (Ceylon) (Table 9): In nasal and ocular rhinosporidiosis in Sri Lanka, Muslims had a disproportionately larger incidence than the other races (Table 9). Nasal rhinosporidiosis is considered to be more prevalent in Muslims because their religious practices "enjoins the thorough washing out of the nose before entering the mosque for prayer, and I am given to understand that this may include the mechanical cleaning of the nostril with the finger" (Karunaratne, 1964), an ideal scenario for the inoculation of finger-borne *R. seeberi* from the communal pond of water used for ablutions before prayer. Kurup (1931) considered that "customs, ways, manners and sanitary conditions" were important in the incidence which was greatest among the Muslims on the west coast of India. Penile or urethral rhinosporidiosis had a higher incidence among Muslims (Tirumurti, 1914) which Kutty and Unni (1969; see also Billore, 1995) attributed to their habit of "rubbing stones on the urethral orifice to remove the last drops of urine". In the present series Muslims predominated with 4.8% over Tamils at 3.4%, contrasting with the former's lower population percentage (Table 9). The only patient with penile/urethral rhinosporidiosis in our series was a Muslim.

The majority of our patients were in agricultural occupations; Karunaratne (1964) noted that 44 % of his patients were paddy cultivators, and postulated a special role of paddy fields as a major source of the pathogen. In the Dry Zone of Sri

Lanka (Fig 1) where most of our patients lived, the predominant agricultural occupation is paddy cultivation. The paddy link as emphasized by Karunaratne (1964) is discussed below.

As in our series where 95% of patients gave a history of regular bathing in lacustrine waters (reservoirs, lakes and rivers), the majority of Indian patients also had a similar history (Billore, 1995) with over 90% of the patients in Tamil Nadu with the highest incidence of rhinosporidiosis, having had a history of bathing in ponds.

The relative percentages of occurrence of the 4 major blood groups in Sri Lanka's population were: A 21%, B 26%, O 43% and AB 5%. The blood group distribution of the cases of rhinosporidiosis paralleled the national figures for blood group distribution in the general population. Except for Group O, data show both in Sri Lanka and India the incidence of rhinosporidiosis parallels the distribution in the normal population, the Sri Lankan data regarding the distribution of other groups in patients differs from that in India in relation to Group AB which has a higher incidence (20%) in India than in Sri Lanka (5%).

Kameswaran in India (1986) examined the blood-group relationship with rhinosporidiosis in 500 patients and noted the following distribution in order of decreasing percentage of incidence: Group O 69.8%, Group AB 20%, Group B 6% and Group A 4.2%. He concluded the significance of the high incidence of rhinosporidiosis in these two groups (O and AB) "is not fully understood". He recorded the national (Indian) blood group distribution as Group A 23%, Group B 34%, Group O 35% and Group AB 8%. The highest percentage of Group O in this series of cases is compatible with the highest incidence

of this group in the Indian general population, although the appreciably high incidence of rhinosporidiosis in Group AB persons (20%) contrasts with the prevalence of Group AB in only 8% of the general Indian population. The prevalence of rhinosporidiosis in Group AB has a different distribution in Sri Lanka (5%) than India (20%) though in the latter country its prevalence in the general population was the lowest (8%). The highest incidence in Sri Lanka was Group O which was also the commonest group in the general population. These differences between Sri Lanka and India occur despite genetic relationships between the Sinhalese and Tamil populations in Sri Lanka and the Indians in Bengal and Gujarat (from where the origins of Sinhalese have been traced) and in South India from where Tamils originated.

Jain (1967) could not draw any conclusions regarding blood group relationships in rhinosporidiosis in India.

It was previously shown in lymphoproliferative (LPR) assays regarding responses of peripheral blood lymphocytes to non-specific mitogens and rhinosporidial antigen *in vitro* that *R. seeberi*-infected patients in this series had demonstrable cell-mediated anergy compared with normal (asymptomatic) persons (de Silva *et al*, 2001), and the depressed LPR was specific to rhinosporidial antigen. Rhinosporidial tissues were also found to have appreciable levels of CD8-bearing lymphocytes as determined by immuno-histochemistry while a proportion of CD8 lymphocytes could be T-suppressor cells, which, at the time of analysis, had no markers for identification.

The LPR finding that antigen (*R. seeberi*) specific immunosuppression was present in patients with rhinosporidiosis, is compatible with the finding of numer-

ous CD8+ lymphocytes in rhinosporidial tissues, although the suppressor sub-type CD8+ cells could not be identified due to lack of markers.

To investigate whether demonstrated CMI anergy was immunologically non-specific or specific to rhinosporidial antigens, Mantoux reactivity was tested in our patients with rhinosporidiosis and compared to the general population using previous data (Pinto *et al*, 1972). Normal (nonimmunized) populations in Sri Lanka who lived in areas where rhinosporidiosis was prevalent, were shown to have a Mantoux-positive rate (percentage) of approximately 65%. Patients with rhinosporidiosis had Mantoux reactivity in only 35%, ($p < 0.001$). CMI anergy to tuberculin PPD in rhinosporidial patients was nonspecific, probably due to general suppression of immune reactivity by *R. seeberi*. Skin testing antigens for *R. seeberi* are not available. One patient with disseminated rhinosporidiosis had a negative reaction to a skin test with soluble antigens obtained from *R. seeberi* from his own tissues by ultrasonic disintegration (Arseculeratne, 1998; unpublished data) indicating CMI anergy to rhinosporidial antigens.

The higher incidence of Mantoux negativity in patients than in the normal population, indicates nonspecific suppression of CMI with rhinosporidiosis, which is contradictory to the antigen specific depression seen with lymphoproliferative assays *in vitro*. The cause of this paradox is unclear. The predominance of CMI anergy in rhinosporidial patients does not indicate that this disease is an opportunistic disease, since there is no evidence in the literature that rhinosporidiosis is more prevalent in patients with immunosuppression. The implication is rather that rhinosporidial antigens may cause immu-

nosuppression as a mechanism of immune evasion (Arseculeratne, 2005). These results suggestive of immunosuppression agree with the findings of the only definitive report on CMI in rhinosporidiosis in India (Chitravel *et al*, 1981) that reported the Leukocyte Migration Inhibition test (LMI, a test of CMI) showed LMI was maximal in patients with disease of less than 9 years duration and that LMI waned beyond that period, suggesting immuno-suppression occurred. Our experiments on mice with rhinosporidial antigen demonstrated immune deviation (Jayasekera *et al*, 2001; Arseculeratne, 2005), compatible with the findings of Chitravel *et al* (1981).

While our data (Arseculeratne *et al*, 2004) from patients in this series showed appreciable anti-rhinosporidial antibody titers in those patients with rhinosporidiosis, there is only one report from India (Chitravel *et al*, 1981) that investigated humoral responses and documented no antibody was detected; our investigations used the same methods and the negativity of the Indian report was attributed to the use of inappropriate antigens.

The finding of higher antirhinosporidial IgG titres than IgM titers in our patients is compatible with rhinosporidiosis of long duration. The appreciable titers in asymptomatic persons were attributed to subclinical sensitization following exposure to *R. seeberi*, by bathing in the same lacustrine waters which are the natural habitat of *R. seeberi* (Kaluarachchi *et al*, 2008). None of these immunological parameters in patients with rhinosporidiosis showed correlations with the sites of clinical disease.

A history of exposure to ground water and the predominant habitat of the patients in the Sri Lankan series in the Dry Zone with numerous lacustrine collections of water (Fig 1) in which the patients habitu-

ally bathe, is compatible with the evidence such waters are the natural habitat for *R. seeberi*. Deposits from the water of the reservoir in the Dry Zone of Sri Lanka are most commonly used for bathing by these patients and showed the presence of *R. seeberi* by *R. seeberi*-specific hybridization probes *in situ* (Kaluarachchi *et al*, 2008).

The higher incidence of ocular rhinosporidiosis in Kandy which is a wet zone of Sri Lanka contrasts with the view that ocular disease occurs especially in dry, dusty environments with dust storms. Kaye's (1938) patient with ocular rhinosporidiosis had no exposure to ground water. Bathing in rivers with suspended sharp spicules derived from sand may explain the occurrence of ocular lesions in persons occupationally or recreationally exposed to river water (Senaratne *et al*, 2007).

Lacustrine ground water, such as lakes, reservoirs, rivers and wet soil in paddy fields is not the only putative source of the pathogen. Karunaratne (1964) found in his series of 225 patients, 44% were paddy cultivators and 42% worked in dusty environments. The differentiation between wet and dry-dusty environments as sources of *R. seeberi*, as related to the occurrence of respiratory and ocular rhinosporidiosis, respectively, is probably unreal because wet paddy fields become dry and dusty with air-borne *R. seeberi* found during the dry seasons. The importance of paddy fields as sources of *R. seeberi* has probably been over emphasized.

The RAPD results indicate the genotypic heterogeneity of *R. seeberi*. This is the first report regarding strains from Sri Lankan patients, and of the genetic heterogeneity in human strains; strains of *R. seeberi* from human and animals sources have been found by other workers to be genetically different (Silva *et al*, 2005).

No previous data from *in vitro* tests are available in the international literature because *R. seeberi* has never been cultured *in vitro*, but data regarding clinical response has been reported mainly for Dapsone, (Arseculeratne and Mendoza, 2005b). It was found that Dapsone reduced recurrence rates and promoted the hosts' defensive responses while causing degeneration of the pathogen. Our *in vitro* data (Arseculeratne *et al*, 2008) which gave the lowest IC₅₀ of 29.7 µg/ml for Dapsone is compatible with the clinical responses of Indian patients to Dapsone, reported by these authors. Anecdotal evidence from elsewhere, regarding the effects of amphotericin B and Ketoconazole, was briefly reviewed (Arseculeratne and Mendoza, 2005b; Arseculeratne *et al*, 2008). The extreme sensitivity of rhinosporidial endospores to destruction/inactivation by heat and by chemical agents including biocides and antimicrobial drugs is remarkable and may partly account for the absence of reports regarding laboratory acquired infections and even for transmissibility between patients and normal persons. In its sensitivity to inactivation, the endospore differs markedly from the endospores of bacteria, a feature that may justify the term "spore-morula" as more accurate for the rhinosporidial endospore, as used by Minchin and Fantham (1906). It has been shown the rhinosporidial endospore is comprised of DNA-containing electron dense bodies that were regarded as the ultimate spore or generative body of *R. seeberi* (Arseculeratne *et al*, 2005a) thus further justifying the name "spore-morula" for the endospore. We would correspondingly agree with Minchin and Fantham (1906) that the electron dense bodies would be more accurately termed "spores" within the spore-morula, hitherto called the "endospore".

ACKNOWLEDGEMENTS

We thank the National Science Foundation, Sri Lanka for a research grant to SNA, the consultant ENT surgeons and ward staff of the General Hospitals of Kandy and Anuradhapura, Sri Lanka, for assistance with the patients and Professor GH Peiris for help with the geographical data.

REFERENCES

- Allen FRWK, Dave ML. The treatment of rhinosporidiosis based on the study of sixty cases. *Indian Med Gaz* 1936; 71: 376-95.
- Arseculeratne SN. Mechanisms of immune evasion in *Rhinosporidium seeberi*. *Bull Sri Lanka Coll Microbiologists* 2005; 1: 56-8.
- Arseculeratne SN, Atapattu DN. The assessment of the viability of the endospores of *Rhinosporidium seeberi* with MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide). *Mycol Res* 2004; 108: 1423-30.
- Arseculeratne SN, Atapattu DN, Balasooriya P, Fernando R. The effects of biocides (antiseptics and disinfectants) on the endospores of *Rhinosporidium seeberi*. *Indian J Med Microbiol* 2006; 24: 85-91.
- Arseculeratne SN, Atapattu DN, Wickremaratne K. Nature and significance of the electron-dense bodies of the endospores of *Rhinosporidium seeberi*: their reactions with MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) and TMRE (tetramethyl rhodamine ethyl ester). *Med Mycol* 2005a; 43: 261-73.
- Arseculeratne SN, Kumarasiri PVR, Rajapakse RPVJ, Perera NAND, Arseculeratne G, Atapattu DN. Anti-rhinosporidial antibody levels in patients with rhinosporidiosis and in asymptomatic persons in Sri Lanka. *Mycopathologia* 2004; 158: 157-69.
- Arseculeratne S, Kumarasiri R, Sumathipala S, et al. *In vitro* susceptibility of the endospores of *Rhinosporidium seeberi* to seven antimicrobial agents. *J Infect Dis Antimicrob Agents* 2008; 25: 135-43.
- Arseculeratne SN, Mendoza L. Rhinosporidiosis. In: Merz WG and Hay RJ, eds. *Topley & Wilson's microbiology and microbial infections. Medical mycology*. 10th ed. London: Hodder Arnold, 2005b.
- Arseculeratne SN, Panabokke RG, Atapattu DN. Lymphadenitis, trans-epidermal elimination and unusual histopathology in Human Rhinosporidiosis. *Mycopathologia* 2001; 153: 57-69.
- Billore OP. Rhinosporidiosis. Delhi: A. I. T. B. S., 1995.
- Cherian PV, Vasudevan A. A case of rhinosporidiosis in the female. *J Laryngol Otol* 1929; 44: 518-9.
- Cherian PV, Satyanarayan C. Rhinosporidiosis. *Indian J Otolaryngol* 1949; 1: 15-9.
- Chitravel V, Sundararaj T, Subramanian S, Kumaresan M, Kunjithapadam S. Cell mediated immune responses in human cases of rhinosporidiosis. *Sabouraudia* 1981; 19: 135-42.
- de Silva NR, Heugel H, Atapattu, DN, et al. Cell-mediated immune responses in human rhinosporidiosis. *Mycopathologia* 2001; 152: 59-68.
- Eriyagama NB, Arseculeratne SN. Further observations on the effects of biocides (antiseptics and disinfectants) on the endospores of *Rhinosporidium seeberi*, as assayed by the MTT-reduction test. *J Infect Dis Antimicrob Agents* 2007; 24: 125-32.
- Farmer BH. Land use lessons learnt in Madras and applicable to the Dry Zone of Ceylon. *Bull Ceylon Geogr Soc* 1956; 10: 9-19.
- Jain SN. Aetiology and incidence of rhinosporidiosis: A preliminary report. *Indian J Otolaryngol* 1967; XIX: 1-21.
- Jayasekera S, Arseculeratne SN, Atapattu DN, Kumarasiri R, Tilakaratne WM. Cell mediated immune responses to *Rhinosporidium seeberi* in mice. *Mycopathologia* 2001; 152: 69-79.
- Kaluarachchi K, Sumathipala S, Eriyagama

- NB, Atapattu DN, Arseculeratne SN. The identification of the natural habitat of *Rhinosporidium seeberi* with *R. seeberi*-specific *in situ* hybridization probes. *J Infect Dis Antimicrob Agents* 2008; 25: 25-32.
- Kameswaran S. Studies in rhinosporidiosis. India: Madurai Kamaraj University Publications Division, 1986.
- Karunaratne WAE. Rhinosporidiosis in man. London: The Athlone Press, 1964.
- Kaye H. A case of rhinosporidiosis on the eye. *Br J Ophthalmol* 1938; 22: 447-55.
- Kurup PK. *Rhinosporidium kinealyi* infection. *Indian Med Gaz* 1931; 66: 239-41.
- Kutty MK, Unni PN. Rhinosporidiosis of the urethra. *Trop Geogr Med* 1969; 21: 338-40.
- Leeming G, Smith KC, Bestbier ME, Barrelet A, Kipar A. Equine rhinosporidiosis in United Kingdom. *Emerging Infect Dis* 2007; 13: 1377-9.
- Mandlik GS. A record of rhinosporidial polypi with some observations on the mode of infection. *Indian Med Gaz* 1937; 72: 143-7.
- Minchin EA, Fantham HB. *Rhinosporidium kinealyi* n. g., n. sp. A new sporozoon from the mucous membrane from the Septum Nasi of Man. *Quart J Microscop Sci* 1906; 49: 521-32.
- Noronha AJ. A preliminary note on the prevalence of rhinosporidiosis among sand workers in Poona, with a brief description of some histological features of the rhinosporidial polypus. *J Trop Med Hyg* 1933; 36: 115-20.
- Pinto MRM, Arseculeratne SN, Urugoda CG, Hemawardena DM. Differential tuberculin testing in rural populations in Ceylon. *Tubercle* 1972; 53: 182-97.
- Satyanarayana C. Rhinosporidiosis with a record of 255 cases. *Acta Otolaryngol* 1960; 51: 348-66.
- Senaratne T, Senanayake S, Edussuriya K, Wijenayake P, Arseculeratne SN. Ocular rhinosporidiosis with Staphyloma formation: the first report in Sri Lanka. *J Infect Dis Antimicrob Agents* 2007; 24: 133-41.
- Silva V, Pereira CN, Ajello L, Mendoza L. Molecular evidence for multiple host-specific strains in the genus *Rhinosporidium*. *J Clin Microbiol* 2005; 43: 1865-8.
- Sudharshan V, Goel NK, Gahine R, Krishnani C. Rhinosporidiosis in Raipur, Chattisgarh: A report of 462 cases. *Indian J Pathol Microbiol* 2007; 50: 718-21.
- Tirumurti TS. *Rhinosporidium kinealyi*. *Practitioner* 1914; 93: 704-19.
- Vucovic Z, Bobic-Radovanovic A, Latkovic Z, Radanovic Z. An epidemiological investigation of the first outbreak of rhinosporidiosis in Europe. *J Trop Med Hyg* 1995; 98: 333-7.
- Wright RE. *Rhinosporidium kinealyi* of the conjunctiva. *Indian Med Gaz* 1922; 57: 82-3.