Development and survival of *Ascaris lumbricoides* eggs under the high-rainfall and humid conditions prevailing in Meghalaya, India

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ABSTRACT

This study investigated the rate of development and survival of *Ascaris lumbricoides*, a gastro-intestinal parasite of man, under the controlled laboratory conditions simulating the ambient climatic conditions that prevail in Meghalaya, a high-rainfall, humid area of India. The objective was to identify the features of environment that were significant predictors of *A. lumbricoides* infections in the area. The data obtained indicate that under the existing climatic conditions, the *Ascaris* eggs deposited on the soil during June to early July could complete their development and reach to infective stage in 48-52 days time. Keeping the temperature constant (28°C), eggs incubated at relative humidity, 80% and 100% showed their complete development to infective stages in 26-27 and 21-25 days, respectively. Slightly acidic soil favoured relatively rapid development of eggs. Survival of infective eggs was 15-18 and 30-56 days at relative humidity 40% and 60%, respectively. At higher relative humidities (80% and 100%) eggs were found to be still viable after 210 days.

It is observed that under the prevailing climatic conditions of the region the *Ascaris* eggs could develop to infective stage only during a short period of time during a year and their survival in the environment would be for a relatively long period of time, which in turn, could pose a continued risk of recruitment of infection among community people throughout the year.

Key words: Ascariasis, *Ascaris lumbricoides*, Epidemiology, Soil-transmitted helminths, Climatic factors, High-rainfall area.

INTRODUCTION

Infections with *Ascaris lumbricoides*, a gastro-intestinal nematode parasite of man, are commonly encountered health problems in many regions of India, where the socio-economic environment is dominated by poverty, poor living status and insufficient sanitation (Awasthi and Pandey, 1997; Paul and Gnanamani, 1999; Chakma et al., 2000). *Ascaris* eggs are passed through in feces of the host in the environment where they undergo the process of development to reach to an infective stage (second-stage larva-L2), which when ingested leads to infection in the host. During their stay in the environment the rate of egg development and also their survival in soil depend upon the ambient climatic conditions, namely-temperature, relative humidity, rainfall, etc. (Gaasenbeek and Borgsteede, 1998; Wagner and Polley, 1999). These factors thus predominantly determine the occurrence and dissemination of *Ascaris* infection in a geographical area. The influence of climatic factors upon epidemiology of ascariasis has been studied under the varying climatic conditions of the world (Velichkin and Troyan, 1984; Larsen and Roepstorff, 1999). However, no information is available on the role of these factors in transmission of ascariasis in Meghalaya that represents a high-rainfall and humid climatic zone of India. The present study was, therefore undertaken, with a view to investigate the possible role of environmental factors, namely-temperature, relative humidity and pH of soil upon the development and survival of *Ascaris* eggs to infective stage so as to draw an inference on their possible role in disseminating ascariasis in the area.

MATERIALS AND METHODS

Study Area

The state of Meghalaya covers an altitude range of 400-1600 m ASL and experiences a monsoon climate. The
average annual rainfall is 400 cm, while in southern parts it is up to 1040 cm, recorded at Cherrapunjee and Mawsynram, the wettest spots of the world (Lynden et al., 1998). The range of temperature is low, varying between a mean minimum of 8°C in winter to a mean maximum of 24°C during summer months. These weather conditions tend to produce a high relative humidity in the environment, particularly during summer months, when it varies between 85 and 90%.

**Experiments**

Three climatic factors, namely-temperature, relative humidity and pH of soil, were selected to see their effects on development and/or survival of *Ascaris* eggs. The experiments were set with the uterine eggs collected from freshly collected live *A. lumbricoides* worms. Approximately 2000±50 eggs were introduced in the petri dishes containing 40 g of sterilized soil in a climatic chamber at various combinations of temperature and relative humidity. The two sets of temperature and relative humidity (R.H.) conditions (i.e., 13°C/64% R.H. and 20°C/83% R.H.) used for incubation of eggs, in general represent the average conditions prevailing in the region between the second and third (April-September) and the last and the first quarters (October-March), respectively of the year. The controls were incubated at 28°C temperature and 90% relative humidity. To study the influence of soil pH on the development of eggs, the temperature and relative humidity was kept constant and eggs were incubated at slightly acidic (pH 5.0), neutral (pH 7.0) and alkaline (pH 9.0) soil. In all the above mentioned experiments, the process of egg development to infective stage was observed regularly at 24 hrs interval. Each time a little amount of soil sample was withdrawn from petri dish and the eggs under development were separated using salt floatation technique (Soulsby, 1982), and observed under a microscope. The infectivity of eggs was confirmed using a mouse bioassay (Wagner and Polley, 1999).

**RESULTS**

The effect of various climatic factors on the rate of development and/or survival of *Ascaris* eggs is summarized in Table 1. The eggs incubated at 20°C temperature and 83% relative humidity showed their development to infective stage between 48-52 days, whereas those at 13°C and 64% R.H. failed to reach the infective stage and degenerated between 60-80 days. Similarly, when incubated at various relative humidities, keeping the temperature constant, the eggs could not reach to infective stage at R.H. 40% and 60% and degenerated between 60-80 days, whereas those incubated at 80% and 100% relative humidity did develop to this stage in 26-27 and 21-25 days, respectively. The soil pH does not seem to have much influence on the egg development (Table 1); however, relatively rapid development of eggs was recorded in acidic soil (pH 5.0). Regarding the survival of infective eggs, those incubated at 40% and 60% relative humidity could survive for 15-18 and 30-56 days, respectively, whereas no mortality was noticed in case of those kept at 80% and 100% relative humidity till a period of 210 days, following which the observations were discontinued.

**DISCUSSION**

This study indicates that the development of *Ascaris* eggs to infective stage in this area is possible only at 20°C temperature and 83% relative humidity. Krasnovos and Zhakangirov (1981), in a field designed experiment, provided an account of forecast of development of *Ascaris* eggs in the 8 districts of Samarkand region of erstwhile USSR; the minimum and maximum time for egg development were established between 27 and 40 days in foothills, and between 173 and 178 days in plain zones, respectively. In another study of similar kind at Herts (U.K.), Stevenson (1979) found that the *Ascaris* eggs introduced in the soil during summer could reach to infective stage in as short as a period of 14 days; however, the development of eggs was possible only between mid-June to August (i.e., a period of about 75 days). Theoretically, it may be speculated that under the ambient temperature of region, *Ascaris* eggs deposited on the soil during June and early July could complete their development and attain the infective stage in 48-52 days (i.e., later half of July to August) and such eggs would play an important role in transmission of infection to the community people. Whereas, the eggs voided July onwards would bear little or no epidemiological significance since these could not complete their development during this period. Regarding the influence of relative humidity on development of eggs, the study indicated that the eggs could complete their development to infective stage in 26-27 and 21-25 days at 80% and 100% relative humidity, respectively. Since the mean relative humidity recorded in this region is above 80% for major part of the year, the eggs would always have potentials to develop and become infective for a considerable long span of time during the year. In the case of soil pH, though no discrete change was
Development *Ascaris* eggs

Table 1. Patterns of *Ascaris* egg development and survival at various climatic conditions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time During (in days) Egg development</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature / Relative Humidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28°C / 90%</td>
<td>22-25</td>
<td></td>
</tr>
<tr>
<td>20°C / 83%</td>
<td>48-52</td>
<td></td>
</tr>
<tr>
<td>13°C / 64%</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Relative Humidity**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td>*</td>
<td>15-18</td>
</tr>
<tr>
<td>60%</td>
<td>*</td>
<td>30-56</td>
</tr>
<tr>
<td>80%</td>
<td>26-27</td>
<td>210+</td>
</tr>
<tr>
<td>100%</td>
<td>21-25</td>
<td>210+</td>
</tr>
<tr>
<td>Soil pH***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>26-30</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>29-34</td>
<td></td>
</tr>
<tr>
<td>9.0</td>
<td>30-42</td>
<td></td>
</tr>
</tbody>
</table>

* Eggs could not complete development and degenerated between 60-80 days
** Incubating temperature 28°C
*** Incubating temperature and relative humidity: 28°C and 90%
+ Observations were discontinued after this period

noticed in the development of eggs to infective stage, yet a relatively rapid development of eggs in acidic soil (pH 5.0) may bear some practical significance, since it corresponds very closely with the pH of soil (5.0-6.0) found in the area under the present study. In a study on sludge decontamination by alkaline treatment at various temperature, Ghigletti et al. (1995) reported that combination of alkalization at a temperature of 30°C causes *Ascaris* eggs to be inactivated. Suggestively, the acidic soil seems to be more favourable for embryonation of *Ascaris* eggs.

With regard to the influence of relative humidity on survival of infective eggs it was noticed that though their survival was only for few days at relative humidity 40% and 60% but at higher humidities (100% and 80%) eggs were found to be viable even after 210 days. Under field conditions it may be speculated that a considerable long survival of infective eggs in the environment would always enhance the risk of their acquisition by inhabitants throughout the year.

Earlier studies in the area related to plausible factors of zoonotic importance have shown that the soils in places of frequent human exposure and also certain vegetables highly contaminated with *Ascaris* eggs could be a potential source of infection to the inhabitants. A high prevalence of *Ascaris* infections in human and pig hosts of the region has also been recorded (Yadav and Tandon, 1989). Further, electron microscopic studies on *Ascaris* recovered from the local pigs showed morphological similarities to those of human origin. In view of this, the cross infection to human subjects with the infective stages of *Ascaris* of pig origin seems a possibility. There are reports indicating towards the role of pigs in disseminating ascariasis to human subjects in the area where the two live in close association with each other (Peng et al., 1996), a situation similar to the area under the present study. It is therefore concluded from this study that under the high-rainfall and humid conditions of the area the developmental and survival potential of *Ascaris* eggs in soil can pose a risk of infection to human subjects.
LITERATURE CITED


Revised : 12 August, 2002
Accepted : 26 August, 2002