*Ceylon J. med. Sci.*, 25 (Nos. 1 & 2 June/Dec.) 1976 pp. 1-5.

# Effect of two Commonly used Nitrogenous Fertilizers on Infective *Ascaris lumbricoides* Ova.

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SUMMARY The effect of urea and ammonium sulphate on infective *Ascaris* ova was studied. It was shown that these two compounds have no ovicidal effect on infective *Ascaris* ova in the concentrations used. Decortication of the ova was observed in the first two concentrations of both test solutions. The motility and the hatchability of the contained larvae were also lowered in the higher concentrations of the test solutions.

## INTRODUCTION

Ascariasis is considered to be a major public health problem in Sri Lanka and is held responsible for a significant proportion of morbidity and mortality in the 1—4 year age group (WHO, 1967). Obvious morbidity and mortality due to ascariasis reflects only a very small part of the impact this disease has on the health of a population (WHO, 1964). In Sri Lanka where most of the children and a large proportion of the adult population are infected, it can be assumed that the soil in the immediate environment is being continuously contaminated with faeces from infected persons.

The development of *Ascaris* ova to the infective stage depends upon : (1) temperature, (2) adequate moisture and (3) sufficient oxygen (Watson, 1960). The ovum which measures 50—70 millimicrons by 40—50 millimicrons consists of an embryo surrounded by a wall with three layers (Belding, 1965 ) ; an inner, delicate, lipoid, vitelline membrane which is highly impermeable and secreted immediately after fertilization, a thick, transparent chitinous layer or the true shell and an outer, coarse, mammilated albuminous coat which is deposited in the uterus and serves as an auxiliary barrier to permeable substances.

*Ascaris* ova are remarkably resistant to various chemical and physical agents. Development takes place at a temperature ranging from 23°C to 32°C. They are very susceptible to desiccation and are quickly destroyed at 65°C or when frozen at —20°C. The outer coat is dissolved by acids and alkalis without affecting the viability of the embryo. Effect of various chemicals on *Ascaris* ova has been carried out. In sodium hypochlorite solutions adjusted to various pH values with acetic acid, optimal ovicidal effect was seen at pH 6.5—6.7 (Grubb and Gonzales, 1969), in aqueous iodine the ovicidal effect was seen at a concentration of 250 ppm (Thitasut, 1961 ; Zaman and Visualingam, 1967) and the ovicidal effect of

night soil was shown by storing the ova in it for eleven days at 34°C (Nishi, 1969). Suspension of non-embryonated eggs in 10% solutions of nitric acid, sulphuric acid, hydrochloric acid, and phosphoric acid does not alter significantly the number of ova reaching larval stages (Rojas, 1971).

In 1969 Hamdi carried out a study of the effect of some nitrogenous fertilizers on fresh and infective ova of *Ascaris lumbricoides* in Egypt, and he concluded that some of these fertilizers, specially ammonium nitrosulphate have dual actions as fertilizers and as *Ascaris* ovicides. Our study was designed to test the effect of two commonly used nitrogenous fertilizers in Sri Lanka, namely urea and ammonium sulphate on infective *Ascaris* ova.

## MATERIALS AND METHODS

Only infective Ascaris ova were used in the study and the following procedure was adopted to obtain them. Fertilized but unsegmented *Ascaris* ova were first obtained by dissecting the uteri of female worms recovered from patients after a vermifuge (piperazine citrate elixir). The ova were kept in Petri dishes, 14 cm diameter, and physiological saline was added just enough to cover the layer of ova at bottom. A few drops of 0.05% formalin were added to prevent excessive bacterial and fungal growth. This culture medium was found to be suitable although Taffs (1964), used a similar procedure but using distilled water. A period of thirty days was taken by the fertilized, unsegmented ova to develop in to the infective stage (second stage larva). The infectivity of these ova was confirmed by oral administration of 10,000 larvae in saline to rabbits and observing the haemorrhagic lesions in the lungs after ten days. Larvae were also recovered from the lungs using Baermann's technique. The concentration of ova was adjusted so that there were 2,600 ova in the infective stage per ml. of saline. Solutions of urea and ammonium sulphate were made in distilled water in the following eight concentrations : 10%, 5%, 2.5%, 1.25%, 0.63% 0.31%, 0.16%, and 0.08%. The tests were carried out in Petri dishes, diameter 4.5 cms. In each Petri dish 1 ml of *Ascaris* ova and 4 ml of the different concentrations of test solutions were added and a correction made to bring them to the appropriate strengths. Since the test solutions were made in distilled water a control with 1 ml of ova and 4 ml of distilled water was used. The Petri dishes were covered with glass lids and the experiment carried out at room temperature. A small drop from each Petri dish was examined under the high power of the microscope, two hourly for the first twelve hours, and six hourly for the next twelve hours. Thereafter the solutions were examined once daily for forty five days for the following signs of viability, (1) motility of the larva inside the ovum (Taffs, 1964, Hamdi, 1969) and, (2) hatchability—gentle tapping with a needle makes the larva come out through a rent in the egg wall in 2—3 seconds. (Hamdi, 1969). The infectivity of the ova at the end of the experiment was checked by feeding them to guineapigs and examining the lungs for haemorrhagic lesions and larvae after 10 days.

TABLE

Changes observed in the ova and the contained Larvae over a period of Forty Five Days.

| Compound | Changes observed in the ova and the contained larvae | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Days | 10% | 5% | 2.5% | 1.25% | 0.63% | 0.31% | 0.15% | 0.08% |
| Urea | 10 | Larvae sluggish, hatchability lowered | | | | | | | |
| | 13 | Decortication of ova | | | No changes observed | | | | |
| | 30 to 45 | Motility and hatchability normal | | | | | | | |
| Amm. sulph. | 10 | Larvae sluggish and hatchability lowered | | | | | | | |
| | 15 | Decortication of ova | | | | | | | |
| | 25 to 45 | Larvae motile and hatchability normal | | | No changes observed | | | | |
| Control | | No changes observed throughout the period of experiment | | | | | | | |

## RESULTS

The results are summarised in the table. The earliest change observed in the ova suspended in 10% and 5% solutions of urea and ammonium sulphate was the lowered motility of the contained larvae. The motility decreased progressively and the larvae found to be extremely sluggish (movements seen only on tapping on the coverslip) in 10% and 5% solutions of urea and 10%, 5% and 2.5% solutions of ammonium sulphates on the 10th day. The lowered motility of the contained larvae continued up to the 25th day in the first three concentrations of ammonium sulphate solutions and up to the 30th day in the case of the first two concentrations of urea. The motility of the larvae increased progressively thereafter and on the 45th day the motility was similar to that seen in the control. The above changes of motility of the larvae were seen only in the first three ammonium sulphate solutions and the first two urea solutions. Throughout the period of the experiment the ova and the contained larvae were normal in the rest of the solutions. The hatchability was also lowered and followed the same pattern as the changes in motility. Lowered hatchability was determined by observing whether the larvae could come out actively through a rent in the egg wall in 2—3 seconds (Hamdi, 1969). The other change observed in the ova was the decortication of the ova suspended in 10% and 5% urea on the 13th day while similar changes were seen in ova suspended in 10% and 5% ammonium sulphate solutions on the 15th day.

The ova from the first three test solutions of urea and ammonium sulphate, and from the control were washed and suspended in distilled water on the 46th day of the experiment. Approximately eight hundred ova containing larvae, taken from each of the above solutions were administered to guinea pigs orally. Haemorrhagic lesions in the lungs and the larvae recovered after 10 days were taken as indication of egg infectivity.

## DISCUSSION

Only ova in the infective stage were used in the study. The criteria for viability and hence the infectivity, looked for during the experiment were motility of the larvae inside the ova and the hatchability.

Taffs (1964) working with *Ascaris suum* which is similar to *Ascaris lumbricoides* in many respects, regarded the motility of the larvae inside the egg shell, once the ova has reached the infective stage (confirmed by animal experiment) as a satisfactory indication of infectivity, although absence of motility did not rule out infectivity. A more reliable test would have been to feed the ova to rabbits or guineapigs and to examine the lungs for haemorrhagic lesions and larvae after ten days. The above procedure was not practicable in our study except at the beginning and at the end of the experiment. We did not observe signs of granulation and vacuolation which would have denoted less of viability and death (Taffs, 1964). Decortication of *Ascaris* ova without impairing the viability of the contained larvae has been observed in acid and alkaline solutions (Rojas, 1971). We observed decortication in the first two ammonium sulphate solutions which could be attributed to the acidic nature of the solutions (tested with litmus paper). The urea solutions were neutral and the decortication observed in the first two solutions could not be explained. It is possible that urea has a direct action on the albuminous coat of the ovum. According to Soulsby (1965), *Ascaris suum* ova, once in the infective stage, has been observed to go into a period of metabolic quiescence. The lowered motility and hatchability could not be attributed to this phenomenon as the above changes were seen only for a period of 15 days in the case of the first three ammonium sulphate solutions and for 20 days in the first two urea solutions. These changes could not have been due to a direct action of the two compounds on the contents of the ova, as the vitelline layer of the egg wall is resistant to most acids and alkalis (Rojas, 1971).

Hamdi (1969) observed that the infective *Ascaris lumbricoides* ova were viable only up to 10 days in 10% urea, 15 days in 5% and 20 days in 2% urea solutions. He also observed hatching of larvae on the 3rd day in the 10% ammonium sulphate solution.

Our results did not confirm Hamdi's work regarding the ovicidal effect and the hatching of ova. In the present study it was necessary to make a correction to overcome the dilution of the test solutions by the addition of ova in saline, and final concentrations were brought to the appropriate strengths. The risk of evaporation of the test solutions was minimised by using larger volumes (5 ml) in covered Petri dishes.

The results of our study show that urea and ammonium sulphate have no ovicidal effect on infective *Ascaris lumbricoides* ova in the concentrations used.

ACKNOWLEDGEMENTS

We are grateful to Dr. C. S. Weeraratna of the Department of Agriculture, Peradeniya Campus for supplying us the fertilizer samples.

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