

SUSCEPTIBILITY OF LABORATORY AND FARM ANIMALS AND TWO SPECIES OF DUCK TO THE MOSQUITO FUNGUS *CULICINOMYCES* SP.

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ABSTRACT. Rats, mice, guinea-pigs, sheep, cattle and two species of wild duck were treated daily with suspensions of the conidia of *Culicinomyces* sp. Treatments were continued for 4 weeks in the case of the ducks and 6 weeks for the other animals.

Suspensions which were pathogenic for

mosquito larvae had no observable effect on the health of the test animals. No significant changes occurred in a range of haematological and biochemical values and at autopsy no macroscopic or microscopic signs of sporulation or tissue invasion by *Culicinomyces* sp. were observed.

INTRODUCTION

Fungi of the genus *Culicinomyces* Couch, Romney and Rao that are pathogenic for mosquito larvae have been discovered independently in Australia (Sweeney et al. 1973) and in the United States of America (Couch et al. 1974). It is not known whether the American species, *C. clavosporous*, and the Australian one are the same. The Australian species is being studied to assess its potential as a biological control agent (Sweeney et al. 1973). Laboratory observations suggest that its host range may be restricted to mosquitoes and other aquatic dipterous larvae within the Division Culicomorpha. Other aquatic insect larvae, fresh water shrimps, and the mosquito-eating fish *Gambusia*, were unaffected by prolonged exposure to viable conidia (Sweeney 1975). A small trial has demonstrated that this fungus kills mosquito larvae in the field (Sweeney and Panter, 1977), but before more extensive trials were done, it was considered necessary to determine its effects on farm animals and water birds which might drink from treated mosquito breeding sites.

This paper reports the results of trials during which infective conidia of

Culicinomyces were administered orally to mice, rats, guinea-pigs, sheep, cattle and 2 species of wild ducks.

MATERIALS AND METHODS

The original Australian isolate of *Culicinomyces* was used. This has been maintained continuously by subculture on nutrient agar since 1972. It was grown in 20 liter culture vessels in a medium consisting of 0.3% beef extract and 0.5% peptone in distilled water with continuous agitation and aeration. After 7-10 days the conidia were harvested by a combination of filtration and centrifugation, suspended in sterile distilled water and stored at -80°C. The infectivity of the different batches for mosquito larvae was confirmed before use.

LABORATORY RODENTS. Five guinea pigs, 5 rats and 10 mice were dosed daily with *Culicinomyces* sp. conidia. This was done by replacing the water supply with distilled water containing 10⁶ conidia/ml in the case of mice and rats and 3.3 x 10⁶ conidia/ml in the case of guinea pigs. Each species of animal was kept in groups of 5 and these 5 had access to a common watering bottle. The volume of conidial suspension supplied each day was calculated from the expected daily water consumption of each of the host species. Observations in the week prior to commencement of treatment provided this

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information. Thus, 30 ml of the suspension was offered daily to 5 mice, 180 ml to 5 rats and 200 ml to the 5 guinea pigs. All animals were fed the same commercial ration *ad lib* for the duration of the experiment. In addition guinea pigs were fed daily with freshly cut grass.

Equal numbers of control animals were kept under the same conditions with the exception that the conidial suspensions were replaced by distilled water. Treatment was continued for 41 days.

Animals were observed closely at least once daily, and were weighed each week. Rats and mice were weighed in groups of 5 whereas guinea pigs were weighed individually. Juvenile animals of each species were used so that a comparison of growth rate between treated and control animals could be made.

RUMINANTS. The sheep and cattle were dosed orally with a suspension of conidia concentrated so that each animal's daily dose contained the number of conidia that would have been consumed had its water contained 10^6 conidia/ml. Thus, 2 Shorthorn cross steers about 5 months of age and 3 adult Merino wethers were dosed orally each day with 50 ml and 20 ml respectively of a suspension of spores that contained 10^8 spores/ml. Treatment was continued for 39 days.

Animals were fed *ad lib* on an oatenslucern chaff mixture for a week prior to and for the duration of treatment. Water was provided *ad lib*. Animals were housed indoors in adjoining rooms of an environment control unit operating at 25°C. Two calves and 3 wethers of similar ages to the test ones were kept as controls.

Animals were identified and weighed at the beginning of the experimental period, and every 7 days thereafter. They were examined clinically each day. Prior to and at 7 day intervals throughout the experiment blood samples were taken for haematological, biochemical and serological examinations. Tests were done to determine values for packed cell volume, haemoglobin concentration, total white cell counts, blood glucose, albumin, globulin, urea nitrogen, calcium and in-

organic phosphorus.

DUCKS. The treatment group consisted of 16 animals—4 males of each species, 5 female black ducks and 3 female wood ducks. The controls were 4 male and 3 female black ducks (*Anas superciliosa*) and 3 male and 3 female wood ducks (*Chenonetta jubata*). The 2 groups were housed separately in deep litter pens. Water consumption was measured daily for each group over a period of 1 week prior to commencement of dosing. During the experiment treated groups were provided daily with distilled water that contained 10^6 spores/ml. All ducks were fed a ration of commercial duck crumbles *ad lib* and water.

Treated and control groups were weighed at the beginning and at the end of the 27 day dosing period. During and after treatment blood samples were collected by venepuncture for a series of clinical pathology tests.

PATHOLOGY. At the conclusion of the feeding period all treated and control animals were killed and autopsied. Portions of small intestine, large intestine, liver, spleen, kidney, lung and heart were selected and preserved in formalin. These were processed by standard techniques, stained with haematoxylin and eosin and examined microscopically.

SEROLOGY. Sera from ruminants and ducks, collected before and after treatment, were tested for agglutinins to *Culicinomyces* conidia. Additional sera from 30 sheep in western New South Wales and 9 human sera were also tested. The latter included samples from 2 laboratory workers who had been in regular contact with the organism for 5 and 2 years.

Serial dilutions of sera were made in phosphate buffered saline, 0.06 M pH 7.4. To 0.5 ml volumes of these dilutions was added a suspension of conidia in 0.5% formal-saline. The optical density of this suspension at $520\mu\text{m}$ was 0.25. A positive control serum was prepared in a rabbit by thrice weekly intravenous inoculation of the spore suspension antigen. The titre of this serum was 2560.

RESULTS

LABORATORY RODENTS. No changes attributable to oral treatment with conidia were observed in any animal. The 5 treated guinea pigs remained healthy and grew at the same rate as untreated controls on the same rations (See Table 1). The treated groups of rats and mice were also healthy throughout the feeding period and grew as well as the control groups.

Table 1. Body weight increases in grams of guinea pigs fed *Culicinomyces* sp. for 41 days

	Treated	Control
Mean	95.2	84.0
Range	18-145	25-179
S.D.	53.67	61.69
$t = 0.3062$ N.S.		

Neither gross nor microscopic lesions were observed in treated animals to suggest multiplication in, or invasion of either the alimentary tract or other organs by *Culicinomyces* sp.

RUMINANTS. Treated cattle and sheep were apparently unaffected by daily oral dosing with *Culicinomyces*. Bodyweight changes in control and treated animals were very similar. No clinical or pathological changes were observed that were attributable to the treatment. Histopathological examinations for evidence of fungal invasion were negative. Haematological and biochemical values were within the normal range.

DUCKS. No adverse effects attributable to ingestion of *Culicinomyces* were observed in either species of duck. Weight changes in control and treated ducks of both species were not significantly different during the trial period. RBC, WBC, PCV, haemoglobin, blood glucose, lipid, bilirubin, urea nitrogen, inorganic P, total protein, albumin, globulin and a series of serum enzyme values in treated animals were not significantly different from those in the controls.

At autopsy no gross lesions ascribable to the effects of *Culicinomyces* were observed. Histopathology revealed no evidence of multiplication or tissue invasion by the fungus.

SEROLOGY. *Ruminants.* All ruminant sera tested had agglutinin titres for *Culicinomyces* conidia. The range was 20-80. Titres in treated animals were no higher than in controls. Levels in 30 sheep sera collected in western New South Wales (320 Km from the laboratory) had similar levels of antibody (Range 10-80, geometric mean 27.01, log S.D. 0.26).

DUCKS. The majority of samples had titres of <10. Samples from 7 birds had titres of 10. These were from four treated birds and 3 untreated controls. Titres were the same both before and after treatment.

HUMANS. All sera tested including those of 2 laboratory personnel had titres of less than 10.

DISCUSSION

The results obtained in these preliminary studies support the available evidence that *Culicinomyces* has a restricted host range. Conidia of the fungus, pathogenic for mosquito larvae, produced no observable adverse effects when fed to laboratory rodents, sheep, cattle or two species of duck.

The normal body temperature of all these animals is at least 38°C and this is considerably above the maximum at which *Culicinomyces* will grow (Sweeney 1978). Another entomogenous fungus, the phycomycete *Conidiobolus coronatus* (= *Entomophthora coronata*) has been isolated from skin lesions in both man and animals (Emmons et al. 1970). It has been isolated from nasal granulomata in horses in Australia (Hutchins and Johnston 1972). An hyphomycete, *Hyphomyces destruens*, is also found in granulomata of horses, (Bridges and Emmons 1961, Johnston and Henderson 1974) but there is no evidence that this fungus is an insect pathogen. Austwick and Copland (1974)

have suggested that *H. destruens* which they induced to form zoospores is a species of *Pythium*—a genus of the *Phycomycetales*.

Whereas our experiments suggest absence of toxicity in a range of mammals and 2 species of birds after continued ingestion of suspensions of conidia 100 times more concentrated than the LD₅₀ for mosquito larvae (Sweeney 1976), we have no evidence to exclude the possibility that the fungus might invade skin wounds or that it may induce allergic responses in animals chronically exposed to it. Furthermore the possibility of infectivity for poikilotherms has not been examined.

The occurrence of agglutinins to *Culicinomyces* in some animal sera suggests either that the organism or one antigenically related to it is common in the environment. Failure of these threshold antibody levels to rise after extensive oral dosing with the organism supports the view that the conidia were either destroyed in the gastrointestinal tract or were voided in feces.

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