

EXPERIMENTAL TRANSMISSION OF CUTANEOUS STREPTOTHRICOSIS  
BY MOSQUITOES

by 1264

HARVEY ROY MATNEY

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## REVIEW OF LITERATURE

Cutaneous streptothricosis, a mycotic exudative dermatitis caused by Dermatophilus congolensis was first described in cattle in the Belgian Congo by Van Saceghem in 1915,<sup>20</sup> but was not recognized in the United States until 1961 when it was reported by Bridges and Romane in Texas cattle;<sup>4</sup> Bentinck-Smith in horses in New York and Vermont;<sup>3</sup> and Dean et al. in deer in New York which was subsequently transmitted to man.<sup>5</sup> Prior to the recognition of this disease in the United States it had been recognized in Africa, Australia, Tasmania, New Zealand, India, Europe, and South America.<sup>1</sup> Since 1961, cutaneous streptothricosis has been reported from Florida,<sup>19</sup> Georgia,<sup>8,18</sup> Iowa,<sup>12,13</sup> Kansas,<sup>9,10,15</sup> Kentucky,<sup>13</sup> and Mississippi<sup>6</sup> in cattle, and in many instances horses.

Many investigators have suggested that prolonged dampness enhances transmission or in some manner promotes appearance of characteristic lesions of the disease and support these views by many reports of unseasonal prolonged rainy spells either preceding or accompanying outbreaks of the disease. Biting insects, as well as spiny plants, have been suggested as a possible vector. Macadam experimentally transmitted the disease with the tick, Amblyomma variegatum.<sup>11</sup> Plowright demonstrated that cattle dipped in gamma benzene hexachloride\* as a preventative measure against ticks did not develop characteristic lesions

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\* Gammexane, Gammatox, Cooper's Gammatox.

while untreated cattle grazing with them did develop lesions characteristic of the disease.<sup>14</sup> Using this same compound in the form of a spray to control flies of the genus Liperosia has been shown to reduce the incidence of streptothricosis.<sup>11</sup> Richard and Pier transmitted the organism between rabbits with the biting fly Stomoxys calcitrans, as well as the common house fly, Musca domestica.<sup>16</sup>

This report describes transmission studies conducted utilizing a biting insect, Culex tarsalis, whose prevalence could be increased by standing water caused by unseasonal rainy periods.

#### MATERIALS AND METHODS

##### THE ORGANISM

The organism used in these experiments were isolates of Dermatophilus congolensis from an equine, and 2 bovines.\* Rabbits were experimentally infected with each isolate in an attempt to increase virulence of the organism. The organism was recovered from the scabs by the Haalstra method<sup>7</sup> and by streaking on defibrinated 10% sheep blood agar containing 1,000 units of polymyxin B sulfate\*\* per milliliter of medium. Typical colonies were removed and recultured on defibrinated sheep blood agar, and maintained on this agar throughout the experiments.

Identification of the organism was ascertained by both macroscopic colony appearance and microscopic morphology of the

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\* Obtained from Communicable Disease Center, Atlanta, Ga.

\*\* Chas. Pfizer & Co., New York.

organism when stained with Giemsa stain for 20 minutes following fixing for 5 minutes in methyl alcohol. Staining was also accomplished using either Methylene Blue or Gram stain.

#### MOSQUITOES

A population of Culex tarsalis was laboratory reared from egg rafts, and from first through fourth instar larvae;\* and were converted from avian to rabbit feeding. The control rabbits were utilized as a blood source to maintain the colony. The adult mosquitoes were fed on alternate days for approximately 3 hours. The rabbits were placed in mosquito cage, forming a collar-like arrangement with the entry sleeve and clamping this snugly around rabbit's neck with forceps. Rabbits did not greatly object to this restraint and minimal conditioning was required. Some type of restraint was required as when rabbits were placed in cage without head restraint, they would turn on feeding mosquitoes and ingest them. The population was maintained throughout the experiments, and mosquitoes for transmission studies were removed from this population and not returned.

Mosquitoes were removed from breeding cage by trapping them in a lamp chimney with screen wire held in place by means of a rubber band covering one end. When mosquitoes were observed within the chimney, the bottom half of a styrofoam hot drink cup was placed over the opposite end. They were conveyed to the

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\* Obtained from Arboviral Disease Section, National Communicable Disease Center, Fort Collins, Colorado. Reared per letter and personal communication of instructions with Dr. Richard O. Hayes, Biology and Control Investigations.

source of infection, and subsequently to experimental animals. Mosquitoes were infected through one end of lamp chimney, then opposite end was placed in contact with the experimental animals.

#### RABBITS

The experimental animals used were mixed breed rabbits weighing 1.5 - 2.0 kg. apparently healthy and free from any skin condition, as determined by daily observation prior to start of experiments and by culturing hair samples and skin scrapings on defibrinated 10% sheep blood agar containing 1,000 units of polymyxin B sulfate per milliliter of medium. Of the 14 rabbits, 2 were randomly selected as controls, the remaining 12 divided randomly into 3 equal groups. Rabbits were individually caged in an enclosed room with a temperature that varied between 21 C. and 35 C. with the exception of the control animals which were both in a single cage. Feed and water was provided ad libitum. Each rabbit in groups I, and II was prepared for experiment by surgically clipping the hair from a 10 cm. by 20 cm. area on the back. In group III, each rabbit was prepared by surgically clipping an area 5 cm. by 5 cm. on the back, and then scarifying the area.

#### EXPERIMENT I.

Eight transmitting chambers (lamp chimneys) containing approximately 50 - 60 female mosquitoes each were placed on agar plates upon which the organism was growing. Chambers were left on agar approximately 1 hour and mosquitoes that did not voluntarily contact the agar were forced to do so by blowing through

screen covered end of chamber. Immediately following removal of enclamped mosquitoes from agar, a sample of these mosquitoes was removed and killed by subjecting them to -29 C. for approximately 5 minutes. Mosquitoes were suspended in saline for approximately 20 minutes after which they were removed and placed on 10% defibrinated sheep blood agar containing 1,000 units of polymyxin B sulfate per milliliter of medium and incubated at 37 C. for at least 2 days. A sample of the supernatant was streaked on like medium and incubated similarly. Following removal of sample, remainder of enclamped mosquitoes were manually held in contact with previously prepared area on back of rabbit. Mosquitoes were allowed to fully feed on rabbit, and released into another cage. The rabbits were placed in this cage on days 2, and 4 following the initial mosquito-rabbit contact. Mosquito egg rafts from these mosquitoes were not returned to original breeding colony.

Following initial mosquito-rabbit contact, and release of mosquitoes within second cage, 50 female mosquitoes were trapped from this cage in a similar manner as from breeding colony, and cultured. Ten of the 50 mosquitoes were partially dissected before being placed on the medium. Partial dissection included removal of legs and digestive tract. Following incubation the plates were observed macroscopically for typical colonies; typical colonies were removed, fixed, and stained for microscopic identification of the organism.

## EXPERIMENT II.

As in experiment I, 8 chambers of 50 - 60 female mosquitoes each were used. These mosquitoes were partially fed upon infected rabbit blood (containing approximately 20 million infective organisms per milliliter of blood) by means of a feeding chamber covered by shaved rabbit skin and maintained at 38 C. by means of a water bath. This was done by mixing sterile heparinized rabbit blood with organisms harvested from defibrinated sheep blood agar plates. When mosquitoes started feeding, the chamber was slowly removed until actively feeding mosquitoes were free. The chamber was rotated slightly and replaced to allow mosquitoes to feed again. This procedure was completed 3 times. When the feeding chamber was removed the fourth time, a sample of mosquitoes were removed for culture. Sample of mosquitoes was then killed, and a portion dipped in disinfectant\* to remove external body contamination; the other portion remained untreated prior to placing on medium and culturing as in experiment I. The chamber was then placed in contact with a previously prepared area on the back of a rabbit and the mosquitoes were allowed to finish feeding. The remainder of the experiment was conducted duplicating experiment I.

The above experiments were then repeated using rabbits previously used in experiment I for experiment II; and the rabbits used in experiment II were utilized as in experiment I.

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\* Amphyl<sup>R</sup>, National Laboratories, Montvale, N. J.

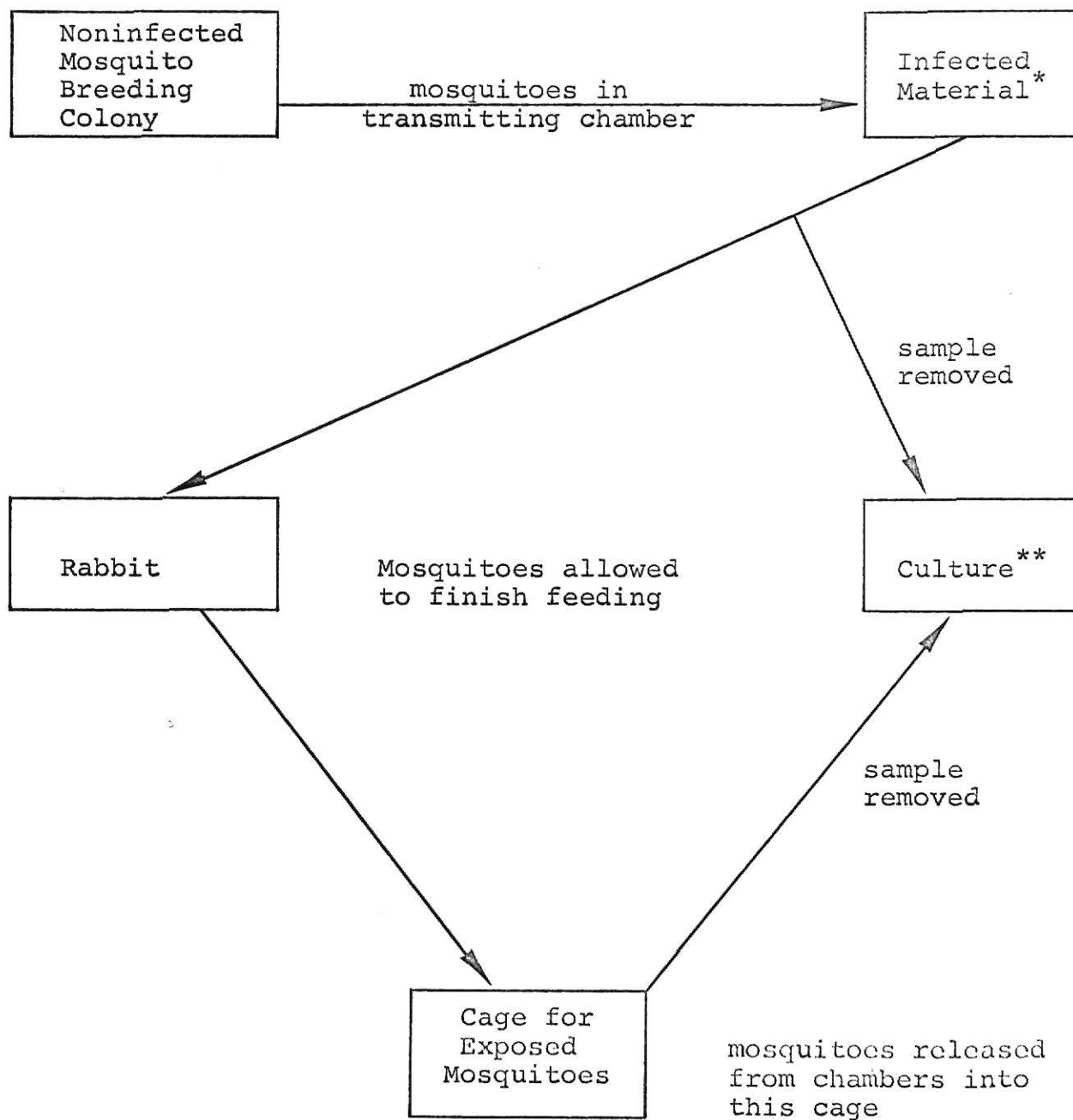


Following completion of these experiments, 4 rabbits were reclipped and shaved, to remove all hair and to scarify an area of the back that was previously exposed. These were then divided into 2 groups of 2 rabbits each and the above experiments repeated.

#### EXPERIMENT III.

One of the group III rabbits was experimentally infected by inoculation of the prepared site with the organism. Four days after experimental infection of rabbit, a mosquito transmitting chamber containing approximately 90 - 100 female mosquitoes was held in contact with the area of infection. When mosquitoes started feeding, the transmitting chamber was slowly moved to interrupt feeding. This was accomplished 3 times, and when the chamber was removed the fourth time, and following removal of a sample of the mosquitoes (cultured as in experiment I), it was held in contact with the prepared area on the experimental animal, where mosquitoes were allowed to complete feeding. Mosquitoes were released into the same cage as in other experiments. With another transmitting chamber containing a like number of mosquitoes, the above was duplicated. Following release of mosquitoes in cage, the rabbits upon which mosquitoes had completed their feeding, were placed in this cage on 2 day intervals 4 times following initial mosquito-rabbit contact and retained in cage until mosquitoes completed feeding. Following initial mosquito-rabbit contact, and after release in the infected mosquito cage, approximately 20 female mosquitoes were recaptured, killed, and

Table I. Flow of Transmission Study of *Dermatophilus congolensis* with Mosquitoes.



\* Infected material was growth on blood agar, infected blood, or experimentally infected case for respective experiment.

\*\* Mosquitoes were killed, and cultured for isolation of the organism.

cultured as in experiments I and II, however, partial dissection of mosquitoes was not performed.

Duplication of this experiment was performed utilizing 2 rabbits of group III.

When scabs were observed they were removed, soaked, and isolation and identification of the organism was attempted.

Numbers assigned rabbits in these 3 groups, as well as colors and results are indicated in Table II under Results.

Route of movement of enchambered mosquitoes from cage containing breeding colony through to release in second cage as followed in the above 3 experiments is shown schematically in Table I.

#### RESULTS

The organism was recovered, isolated, and identified from partially dissected mosquitoes untreated by disinfectant following feeding on infected blood (experiment II). The organism was not recovered from either the intact mosquitoes, or the partially dissected mosquitoes following contact with growth on agar, or feeding on experimentally infected case.

The organism was recovered, isolated, and identified from the experimentally infected rabbits in experiment III.

Rabbits (Table II) used in the above experiments were observed daily for 30 days following final mosquito-rabbit contact. None of the experimental animals developed lesions characteristic of the disease from which the organism could be isolated and identified. The 2 experimental animals in experiment

Table II. Experiments, Rabbits Utilized, and Results.

Experiment Number	Rabbit Number	Color	Infection Observed
Controls	1	White	None
	2	White	"
<hr/>			
I. First Trial	3	Brown	None
	4	Black	"
	5	Black	"
	6	Calif.	"
<hr/>			
Second Trial	7	Black	None
	8	White	"
	9	White	"
	10	White	"
<hr/>			
Scarified Trial	3	Brown	None
	6	Calif.	" *
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II. First Trial	7	Black	None
	8	White	"
	9	White	"
	10	White	"
<hr/>			
Second Trial	3	Brown	None
	4	Black	"
	5	Black	"
	6	Calif.	"
<hr/>			
Scarified Trial	4	Black	None
	8	White	"
<hr/>			
III.	11	Black	Positive**
	12	Black	None*
	13	White	Positive**
	14	White	None*

\* Scabs observed were probably the result of razor nicks, isolation of organism was unsuccessful.

\*\* Experimentally Infected Case.

III developed scabs probably as a result of razor nicks, however, isolation of the organism was unsuccessful.

#### DISCUSSION

Culex tarsalis was selected for use in these transmission studies as it is one of the insects whose prevalence could be greatly increased during seasons of greater than normal amounts of rainfall. Other investigators have suggested the mosquito could be responsible for transmission of this disease.<sup>17</sup> Following a blood meal the female mosquito lays eggs formed in rafts in still standing water as could be found in and around many barn lots or feedlots. The mosquito is a persistent biting insect, normally feeding at dusk, and is a native pest throughout most of the United States.

Moistening the skin of a clipped rabbit proved to be an unsuccessful feeding area, as the mosquitoes refused to feed on the area. The preference of mosquitoes to feed upon the darker, rather than the lighter colored rabbits was observed during the first two experiments. Mosquitoes preferred the healthy clipped areas over the areas that were unclipped, shaved, or scarified. The mosquitoes used were laboratory reared, and no feeding periodicity was observed, and most transmission attempts were conducted during the afternoon.

Recovery of the organism from the digestive tract of mosquitoes fed on infected blood and the fact that mosquitoes do not regurgitate digestive tract contents on successive feedings could be a reason for lack of transmission. Another probable reason

for lack of transmission in this study was that of not wetting the scabs on infected rabbits in experiment III. Richard and Pier were unsuccessful when fly transmission was attempted from dry lesions.<sup>16</sup> The lack of transmission is in agreement with Roberts, who reports virtually no zoospores are detectable at the surface before the scabs have been soaked, infected animals probably do not become a serious source of infection until the lesions are wet.<sup>17</sup>

Previously mentioned was the fact that this disease has been transmitted to man by direct contact with an infected deer.<sup>5</sup> During the course of this study, the investigator developed a small 3 mm. by 3 mm. furuncle on the left forearm. Isolation of Dermatophilus congolensis from the scab removed from this furuncle was accomplished by streaking on 10% defibrinated sheep blood agar with inhibitor. Positive diagnosis was made from microscopic examination of stained slides made from typical colonies as well as from scab material. Pruritis was evident, however, this could have provided route of entry for the organism. Recovery was uneventful, no treatment was utilized.

Probably one of the greatest contributions to understanding the pathogenesis of this disease would be the discovery of the reservoir of the agent, whether or not it is an opportunist saprophyte on the skin of the to be infected animal, and if so how it reaches this animal from its natural reservoir. Consideration could be given to the utilization of the canine as a laboratory animal, as Austwick reported this disease has been

isolated from crust-like lesions on the ears of a wild fox.<sup>2</sup>

#### CONCLUSIONS

Culex tarsalis did not transmit Dermatophilus congolensis from growth on sheep blood agar, infected blood, or experimentally infected rabbits to susceptible rabbits under laboratory conditions. From observations noted herein it is considered unlikely that this mosquito could transmit cutaneous streptothricosis under natural conditions because: 1) It is principally an avian feeder, and if present, would prefer feeding upon an avian species; 2) It will feed upon healthy, preferably glabrous, areas of the infected animal, almost refusing to feed from scarified or infected areas; and 3) It will not feed on a wetted, or soaked area which would be necessary for release of zoospores.

#### SUMMARY

Dermatophilus congolensis, the etiological agent of Cutaneous Streptothricosis was not transmitted to rabbits by the mosquito Culex tarsalis. Experimental transmission was attempted from active growth on blood agar, infected rabbit blood, and experimentally infected rabbits.

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Transmission studies were conducted using the mosquito, Culex tarsalis, as an insect vector in an attempt to transmit Dermatophilus congolensis, the causative agent of cutaneous streptothricosis. Mixed breed rabbits were used as laboratory animals. Three experiments were attempted from active growth on sheep blood agar; from artificially infected rabbit blood; and from experimentally infected rabbits. Mosquitoes were allowed to partially feed on contaminated material as a source of infection, their feeding then interrupted, sample removed for culture, and chambers in which they were contained conveyed to prepared areas on the backs of rabbits on which they were allowed to complete their feeding.

Following completion of feeding, mosquitoes were released into a cage, and another sample removed. Both samples were killed by chilling, placed on sheep blood agar containing an inhibitor and incubated for isolation of the organism. The organism was isolated from partially dissected mosquitoes following feeding on infected blood. Attempts to isolate the organism from other entire and partially dissected mosquitoes was unsuccessful.

Lesions characteristic of the disease were not observed on any of the experimental animals. The organism was isolated and identified from rabbits experimentally infected. The organism was isolated and identified from a small lesion on the author's left forearm. The organism may have been introduced by scratching in response to pruritis of an existing insect bite.

From preferences observed regarding feeding habits of Culex tarsalis, it is considered that transmission of cutaneous streptothricosis would be improbable by this vector under natural conditions.

Through further investigation, if the natural reservoir of Dermatophilus congolensis could be discovered, the pathogenesis of cutaneous streptothricosis, as well as the means of transmission could be more readily understood.