RESERVOIR, PATHOGENESIS, AND TRANSMISSION STUDIES OF DERMATOPHILUS CONGOLENSIS VAN SACEGHEM

by

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TABLE OF CONTENTS

INTRODUCTION..... 1

REVIEW OF LITERATURE..... 3

MATERIALS AND METHODS..... 12

Identification of Organism..... 12

Animal Reservoir Studies..... 12

Collection of Skin Specimens..... 12

Microbiology..... 13

Blood Study..... 13

Pathogenesis Studies..... 13

Organism Source..... 13

Experimental Design..... 14

Rabbit Experiment..... 14

Microbiology..... 16

Histopathology..... 16

Necropsy Data..... 16

Calf Experiment..... 17

Microbiology..... 18

Blood Studies..... 18

Transmission Studies..... 18

Fly Examination..... 18

Calf Transmission Study..... 19

Organism Source..... 20

Fly Exposure..... 21

Microbiology..... 21

RESULTS..... 22

Animal Reservoir Studies..... 22

External Examination..... 22

Blood Study..... 22

Pathogenesis Studies..... 22

Rabbit Experiment..... 22

Histopathology..... 24

Necropsy Data..... 24

Calf Experiment..... 24

Temperature Readings and Blood Studies..... 24

Transmission Studies..... 25

Fly Examination..... 25

Fly Transmission of Dermatophilus congolensis to Calves.. 25

DISCUSSION..... 29

ACKNOWLEDGMENTS..... 35

LITERATURE CITED..... 36

INTRODUCTION

Dermatophilus congolensis Van Saceghem an organism of the Order Actinomycetales, is the etiologic agent of cutaneous streptothricosis. This disease has been described as an exudative dermatitis with extensive scab formation. It was first described by Van Saceghem in 1915 in the Belgian Congo where he had observed it for several years (Austwick, 1958). The disease affects several species of animals of which, economically, cattle are the most important. In Africa, a serious economic loss as a result of hide damage together with debilitation leading to death has been attributed to the disease (Macadam, 1961).

The incidence of streptothricosis has been found to increase during the wet season and decrease during the dry season. This variation led many investigators to conclude that high humidity and constant wetting of the skin were responsible for an increase and spread of lesions. Others thought the increase in tick and biting insect population found during the wet season was of more significance than the high humidity and constant wetting (Ainsworth and Austwick, 1958).

The number of reported cases in the United States have been few, and it was not until 1961 that the first positive diagnosis was reported (Bridges and Romane, 1961). The disease was seen in cattle in Kansas several years before it was identified (Kelly et al., 1964). It was observed most frequently in the Shorthorn breed and was commonly referred

to as "Shorthorn Disease."

Apparently the incidence of streptothricosis has increased in the central United States, although this may be a result of the increased interest and knowledge of the disease.

Because of the contradictions found in the literature regarding the reservoir and vectors of streptothricosis, together with the difficulty encountered in reproducing the disease, this investigation was undertaken with three purposes: (1) to determine if clinically normal cattle from infected and non-infected herds serve as a reservoir for the causative organism D. congolensis, (2) to determine the role of Stomoxys calcitrans as a vector in the transmission of D. congolensis from infected to normal calves and (3) to determine if the route of infection influenced the pathogenesis of the organism.

REVIEW OF LITERATURE

The causative organism of cutaneous streptothricosis was first identified in scab material from infected animals by Van Saceghem in 1915. He named the organism Dermatophilus congolensis and the disease "Dermatose contagieuse (Impetigo contagieux)," thinking it was transmissable by contact. He described the disease as having lesions which consisted of raised circumscribed crusts on the skin composed of epidermal cells and serous exudate with embedded hairs. The condition was local at first but sometimes became progressive and resulted in death (Austwick, 1958).

Van Saceghem described the organism in scab smears as occurring in short lengths of branching mycelial filaments, 1.4 microns to 1.8 microns wide. The mycelia divided both transversely and longitudinally to form cocci. In culture Hudson described the organism as first appearing as a dense growth of branching and tapered hyphae 1 to 2 microns wide. As the organism became older, the hyphae widened to 3 to 5 microns and divided both longitudinally and transversely producing up to eight cells across. The hyphae then broke up into motile cocci (Ainsworth and Austwick, 1958).

A nonspecific dermatitis was reported along the Zambesi River, in Portuguese East Africa, Nyasaland, Northern Rhodesia, and German East Africa by Griffiths (1918). He reported that Van Saceghem identified

a similar disease clinically by five signs: "(1) formation of crusts over which the hairs were erect; (2) rapid spread over the animal: (3) complications which might bring about death; (4) young animals and adults affected to the same degree; (5) a seasonal affection which was observed in the acute form during the rainy season."

Armfield (1918) reported a dermatitis in cattle in Northern Rhodesia which was frequently associated with follicular mange. Photographs of affected animals described as having "chronic follicular mange" accompanied the report and they resembled animals affected with cutaneous streptothricosis. He observed that the common sites of infection were the loins, on the udder of cows, on the scrotum of bulls, and between the thighs. There seemed to be no interference with health until the extent of skin involved disturbed the normal physiology of skin function.

A contagious impetigo of cattle was reported by Hornby (1920). He thought it was the same disease that Armfield had described and also cited the names of other investigators who had reported a similar condition. Lane in Rhodesia reported it as "Senkobo Skin Disease" in 1915, Curson in 1916, called it "Saria" and Hutchins in 1919 reported a similar disease caused by a "streptothrix."

Hornby considered European breeds of cattle to be more susceptible than native stock and reported the disease would assume an epizootic form in European cattle. He further pointed out its seasonal nature, finding new cases in the wet season and regression to a chronic state with no new

cases in the dry season. The lesions were first noticed on the backs of cattle in the form of "paint brushes" which consisted of matted hairs. Later elevations in the skin occurred and wart-like scabs were produced. If the disease spread, lesions developed on the sides of the body, the perineal region, the axillas, inside the thighs, and on the shanks. The udder or scrotum and belly were also involved. Hornby made one of the first references to ticks as a vector of streptothricosis. He reported that both Lane and Armfield suggested a tick-born virus could be the cause of the disease but the appearance of the first lesions on the back made this idea difficult for Hornby to believe because the back was not a common site for ticks.

Hornby demonstrated D. congolensis as described by Van Saceghem and reported that it did not grow deeply in the skin and the hairs within the scabs were never attacked. His conclusions concerning the seasonal occurrence were that the rainy season provided the humidity and temperature necessary for the propagation of the organism. Since the back of cattle was most often exposed to the rain, this was the natural site for the appearance of the first lesions. Lesions of the axilla and perineum probably were a result of tick bites. He further described the appearance of lesions around the mouth of suckling calves and attributed these to the added moisture from the milk in this area.

According to Austwick (1958), the first use of the term "streptothricosis" for the disease name was made by Henderson in 1927. This name

apparently resulted from the earlier implication by Hutchins that a "streptothrix" was the etiologic agent. Streptothricosis has been used since that time for the name of the disease in cattle, goats, horses, elands, zebras, and donkeys.

Bridges and Romane (1961) reported the first known incidence of cutaneous streptothricosis in the United States. Two calves from separate herds in Texas were involved.

Dean et al. (1961) reported the disease in a deer in New York and described four human cases of pustular dermatitis resulting from contact with the infected deer. The causative organism was found to be Dermatophilus sp.

Streptothricosis was reported in 10 horses from New York and from 5 horses in Vermont in 1961; and the authors concluded from the histories of the cases that the organism was probably present on the skin and became infective when the hair coat was wet over long periods of time (Bentinck-Smith et al., 1961).

Pier et al. (1963) diagnosed cutaneous streptothricosis in Holstein-Friesian cattle on an Iowa farm. All 17 animals in one pasture were infected. The cattle had been observed to stand in mud and water for long periods of time and the majority of the lesions were found on the flexor surface of the legs again suggesting the moisture damage to the skin permitted entrance of the organisms.

Cutaneous streptothricosis was reported in Kansas cattle in 1964,

and because the majority of cases occurred in the Shorthorn breed, a genetic involvement was suggested. Most of the affected animals were spring calves which were found to be infected in the fall at weaning time (Kelley et al., 1964).

Two diseases which occur in sheep have similar causative organisms but these originally were classified as different genera. In 1928, a skin condition of unknown etiology (called "lumpy wool") was described by Bekker in sheep in South Africa. The same year Seddon in Australia observed fungus-like organisms in scabs from sheep with a similar disease and called it "mycotic dermatitis." The causative organism was named Actinomyces dermatonomus by Bull in 1928, (Austwick, 1958).

Albiston (1933) in his report of the first incidence of streptothricosis in cattle in Australia, noted the morphological similarity between the organisms found in emulsified scabs from a calf with dermatitis and those causing mycotic dermatitis in sheep. He reported the disease as "Mycotic Dermatitis in the Calf."

Hudson (1937) demonstrated that the causative organisms of "lumpy wool" and streptothricosis were closely related but reported that the organism of cutaneous streptothricosis would not produce "lumpy wool" in sheep.

A second disease of sheep known as "strawberry foot rot" or "proliferative dermatitis" has a similar etiology. It was first considered to be of viral origin because of its clinical similarity to contagious

pustular dermatitis.

Abdussalam and Blakemore (1948) worked with scab material from cases of strawberry foot rot in sheep. In their studies they observed a filamentous organism resembling Fusiformis necrophorus from the exudate of 4-day lesions produced by intradermal inoculation of rabbits. They were unsuccessful in attempts to isolate the organism.

Harriss (1948) assumed the etiological agent to be a virus and found that man was susceptible. Inoculation of a human hand produced an infection characterized by scab-like lesions and intense itching. The lesions lasted for thirty days.

The bacterial etiology of strawberry foot rot was first suggested by Thompson in 1954, when he isolated an organism he considered to belong to a Rhozobium sp. His description of the organism was very similar to that of Actinomyces dermatonomus (Thompson, 1954). Thompson's original isolate was redescribed and named Polysepta pedis (Thompson and Bisset, 1957).

Austwick (1958) proposed the nomenclature of the three genera be changed so as to place them all in the genus Dermatophilus with their respective species names remaining the same: Dermatophilus congolensis, Dermatophilus dermatonomus, and Dermatophilus pedis. Gordon (1963) suggested the three species may be variants of one species.

The implication of moisture of high humidity, insect bites, tick bites, and laceration by inanimate objects, in combination or separately

as predisposing factors is seen throughout the literature.

Roberts (1963a) reported on the properties of motile spores of D. dermatonomus and found that their greatest viability was during the first hours after their release from their mycelia. He suggested that the probability of susceptible skin areas becoming infected would be high when sheep were wet from rain and even greater if some vector was available to transmit the spores from infected to susceptible sheep.

Roberts (1963b) further found that the spores would emerge from the scab only when it was wet and before the spores had lost, through age, their capacity for motility. He conducted soil studies and found that soil from infected premises was not infective for skin of susceptible sheep. He also found that soil contaminated with scab material remained infective for 4 months (longest period tested) if kept dry but lost its infectivity in the wet state. He concluded that although contaminated soil might cause some infections, the survival of the organism and initiation of new cases was more likely due to the presence of active cases of mycotic dermalitis in the flock.

The effect of relative humidity in experimentally infected animals was reported by Chodnik (1956) and Macadam (1961). Chodnik found the lesions commenced to spread after 7 to 10 days in a local moist chamber and disappeared a few weeks after the end of the experiment. These lesions recurred during the next rainy season. Macadam found that experimentally infected animals in a high relative humidity (90%) spontaneously healed

in about two weeks but those held at a low relative humidity (13.5%) did not heal for three to four weeks.

Macadam (1964) reported the effects of high relative humidity (92-93%) on 4 naturally infected cattle which had severe lesions of streptothricosis. Two animals completely recovered, one almost recovered and the fourth had an increase of "paint brush" lesions and died. He stated that the incidence of streptothricosis in cattle in Nigeria was around 0.5% in the dry season and rose to 50% during the rainy season.

In Nyasaland, Zlotnik (1955) described lesions of streptothricosis that appeared first along the backs and loins of cattle. He attributed this to the scratches and lacerations caused by the low branches of trees because most of the lesions were located along these scratches. He felt this suggested that the organism was either a saprophyte of the skin or of vegetation. To further substantiate his opinion, he cited an instance in which cattle kept in a paddock devoid of trees failed to develop lesions of streptothricosis.

Plowright (1956) observed that the areas of the skin most severely attacked by ticks often had the first noticeable lesions of streptothricosis. These areas were the axilla, groin, and scrotum or udder. The reaction to the tick hypostome was severe, resulting in inflammation and edema; abscess formation resulted when ticks attached in clusters. Plowright stated that to prove tick transmission it would have to be shown that the organism did not exist on the skin. Plowright reported

that Wilson's investigations of Amblyoma variegatum revealed only a few male ticks feeding during the dry season. As the rainy season commenced and relative humidity increased, many feeding females were observed.

Macadam (1961) removed A. variegatum from infected cattle and placed them on the ears of rabbits. One positive transmission was obtained. In addition he placed ticks and cultures of D. congolensis on cattle ears. Lesions became apparent in about a month and persisted for about three months. They were typical of the lesions found in the axilla and groins of streptothricosis infected cattle. He noted during this investigation that large numbers of S. calicitrans and Lyperosia spp. attacked the backs of cattle and suggested they might be responsible for "paint brush" lesions on backs of infected cattle.

Macadam (1964) ground A. variegatum and boiled them for five minutes to extract the salivary toxin. He added the toxin to cutaneous scarification sites with cultures of D. congolensis. The skin lesions persisted about a week longer than the lesions produced without the toxin.

Investigations with cattle experimentallyinfected with streptothricosis and exposed to S. calcitrans were carried out by Macadam (1964). He concluded that the organism could be a saprophyte of the skin or that flies could mechanically transmit the infection from one area of the skin to another.

MATERIALS AND METHODS

Identification of Organism

Throughout all portions of the investigation (reservoir, pathogenesis and transmissions studies) identification was based on macroscopic colony appearance and microscopic morphology of the streptothricosis organism.

Animal Reservoir Studies

Two approaches were used to examine clinically healthy cattle for the presence of D. congolensis. An external examination was made by collecting skin scrapings, skin debris, and hair, for cultural examination. In addition, from a second group, individual blood samples were obtained for cultural examination.

Collection of Skin Specimens. Skin scrapings, skin debris, and hair were collected from 9 Shorthorn calves originating from two separate farms where cutaneous streptothricosis had been previously diagnosed. At one of the farms 60 calves were restrained and examined grossly for evidence of lesions. Material for microbiologic examination was collected from 7 animals which had roughened hair coats and scurfiness of the skin. The other 2 calves examined were submitted to the Kansas State University Research Farm for study and microbiologic examination.

Material for microbiologic examination was collected from 15 calves

originating from farms where cutaneous streptothricosis had not been known to exist.

Microbiology. The specimen material was ground using a sterile mortar and pestle and sterile sand. The ground material was inoculated onto 10% sheep blood agar plates containing 1000 units of Polymyxin B Sulfate[1] per ml. of medium and incubated at 37 C for 7 days. Polymyxin B Sulfate at this concentration aided in reducing bacterialand fungal contaminants and allowed the slow-growing colonies of D. congolensis to develop before the blood agar plates were overgrown with contaminants (Kelley et al., 1964).

Blood Study. Blood samples were obtained aseptically from 23 cattle of different ages and breeds. This blood was streaked on 10% sheep blood agar plates and incubated at 37 C for 7 days. These cattle were from a known infected area.

Pathogenesis Studies

This portion of the investigation was undertaken to determine if routes of inoculation other than topical would result in the appearance of streptothricosis organisms in scabs formed by scarification at unrelated sites. Another objective was to see if such lesions would develop into a persistent, spreading infection of streptothricosis.

Organism Source. The culture of D. congolensis used was isolated

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[1]Chas. Pfizer Inc., New York, New York.

from scab material from a clinical case of streptothricosis.[2]

A 5-day nutrient broth culture of the organism was used as the inoculum. It was streaked on 10% sheep blood agar plates to check for viability and purity on the day of animal inoculation.

Experimental Design. Eighteen rabbits and 8 calves were used. Three rabbits were inoculated intradermally, 3 subcutaneously, and 13 intravenously. Eight calves were inoculated intravenously.

Rabbit Experiment. The 18 rabbits were divided into 4 groups. Group I consisted of 6 rabbits (1, 2, 3, 4, 5, and 6). A 5 cm X 8 cm area of skin on the back of each rabbit was shaved and scarified on the day of inoculation. Scarification and inoculation were performed with the rabbits under ether anesthesia. Rabbits 1 and 2 were inoculated intradermally with 3/4 ml. of culture, rabbits 3 and 4 were inoculated subcutaneously with 3/4 ml. of culture and rabbits 5 and 6 were inoculated intravenously with 3/4 ml. of culture. Intradermal and subcutaneous inoculations were made on the right side of the body of each rabbit. Intravenous inoculations were made in ear veins. External application of xylene was used to congest the ear veins.

Five days following scarification and inoculation, the scab material was collected from the back of each rabbit for microbiologic examination.

Group II was used to further substantiate the findings of Group I.

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[2]Culture confirmed by P. K. C. Austwick of the Central Veterinary Laboratory, Weybridge, England.

Three rabbits (7, 8, and 9) were utilized. They were prepared identically to Group I. Rabbit 7 was inoculated intradermally, rabbit 8 was inoculated subcutaneously, rabbit 9 was inoculated intravenously. In addition rabbit 6 of Group I was rescarified but not reinoculated.

Five days following scarification and inoculation, the scab material formed was collected from the back of each rabbit for microbiologic examination.

Group III consisted of 7 rabbits (10, 11, 12, 13, 14, 15, and 16). This group was examined prior to inoculation to determine if D. congolensis was present as a saprophyte on the skin. This was accomplished by shaving and scarifying a 5 cm.X 8 cm.area of skin on the back of each rabbit. Five days following scarification, the scabs on the back of each rabbit were collected for microbiologic examination. Two days following removal of those scabs the seven rabbits were inoculated intravenously with 3/4 ml. of the culture.

All rabbits were rescarified on the original areas at various time intervals. Rabbits 10 and 11 were rescarified on the day of inoculation; rabbits 12 and 13 two days following inoculation; rabbits 14 and 15 four days following inoculation; rabbit 16 six days following inoculation. Five days following rescarification, the scabs from the back of each rabbit were collected for microbiologic examination. A skin biopsy from the scarification site, measuring approximately 0.5 cm. X 2 cm. in size was removed from each rabbit and placed in 10% formalin for

histopathologic examination.

Group IV consisted of 2 rabbits (17 and 18). They were examined for the presence of D. congolensis prior to inoculation as described for Group III. The day the initial scab material was examined, the same areas were rescarified and the rabbits were inoculated intravenously with 3/4 ml. of culture. Five days later, the scab material was removed for microbiologic examination. Skin biopsies were taken as described for Group III.

Microbiology. One portion of the scab material collected from the back of each rabbit was ground and inoculated on 10% sheep blood agar plates containing 1000 units of Polymyxin B Sulfate per ml. of medium. Another portion of the scab material was examined by soaking in sterile saline and smearing on glass slides. The slides were fixed in methyl alcohol for at least 5 minutes and stained with Giemsa stain for at least 15 minutes. The smears were examined with the oil immersion objective (1200X).

Histopathology. Skin biopsies from rabbits of Groups III and IV were each sectioned twice at three different levels. Three sections were stained with hematoxylin and eosin and three with Grocott's fungus stain.

Necropsy Data. Rabbits 5 and 6 were scarified 50 days following intravenous inoculation and examined for lesions. Portions of liver and kidney from rabbit 6 were cultured on 10% sheep blood agar plates containing

1000 units of Polymyxin B Sulfate per ml. of medium. Portions of liver and kidney tissue from rabbit 6 were fixed in 10% formalin and stained with hematoxylin and eosin and Gram's stain.

Rabbits 10 and 18 died as a result of ether anesthesia on the day the skin biopsies were taken (5 days following inoculation and scarification). Rabbit 11 developed a secondary infection at the biopsy site and died 10 days later. Complete necropsies were performed on these 3 rabbits.

Calf Experiment. The 8 calves used in the pathogenesis study consisted of 2 Herefords and 6 Shorthorns. They were approximately 6-10 months of age and weighed 300-450 pounds each.

An area 10 cm. X 15 cm. on the back of each calf was examined for D. congolensis prior to intravenous inoculation as described for the rabbits of Group III. The calves were rescarified on the original 10 cm. X 15 cm. site and inoculated intravenously with 10 ml. of the nutrient broth culture previously described. The scabs were removed 5 days later for microbiologic examination.

In addition, rectal temperatures were recorded and blood studies were conducted on the last 3 calves (335, 369, and 371). These latter examinations were incorporated into the experiment because of negative results in the first 5 calves. The blood and temperature studies were made 11, 6, and 3 days prior to intravenous inoculation, on the day of inoculation, and 1, 2, and 4 days following inoculation. The rectal

temperatures were recorded at the same time each day (8:30 A.M.).

Microbiology. The same methods were employed for culturing and microscopic examination as was described for rabbit pathogenesis studies.

Blood Studies. Disodium ethylenediaminetetraacetate[3] was used as an anticoagulant. The following tests were run: packed cell volume, hemoglobin, total leucocyte and differential leucocyte numbers.

Transmission Studies

This portion of the investigation was divided into two parts. The first was concerned with isolation of D. congolensis either externally, internally, or both, from S. calcitrans fed on sheep blood inoculated with cultures of the organism. The second was concerned with the ability of S. calcitrans to transmit D. congolensis to normal calves from naturally infected calves or artificially infected calves. Scarification on both artificially infected and non-infected calves was deep enough to produce frank hemorrhage. Because of the heavy winter haircoat covering the body of the calves, the shaven and scarified areas were more attractive to the feeding flies.

Fly Examination. Approximately 150 adult S. calcitrans were placed in two boxes 8" X 8" X 8" constructed of fly screen. Five hundred ml. of defibrinated sheep blood was mixed with 50 ml. of a 5-day nutrient

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[3] EDTA, Cambridge Chemical Products, Inc., Dearborn, Michigan.

broth culture of D. congolensis. Surgical cotton was soaked with this mixture and placed on top of the boxes. The boxes were then placed in a room maintained at 80 F. The blood-culture mixture was added twice daily to the cotton.

After the flies died, 25 were examined for D. congolensis by streaking their bodies on 10% sheep blood agar plates containing 1000 units of Polymyxin B Sulfate per ml. of medium. In addition 25 flies were sterilized externally by placing them in a 1:500 alcoholic solution of bichloride of mercury for 30 minutes (Stirrat et al., 1954). They were then washed in two separate solutions of sterile saline and ground by sterile mortar and pestle. The ground material was inoculated on 10% sheep blood agar plates containing 1000 units of Polymyxin B Sulfate per ml. of medium.

To further reduce contaminant bacteria, 100 flies were dried over concentrated sulfuric acid for 9 days. Twenty-five were examined externally and 25 were examined internally as described above. The remaining 50 flies were ground and dried an additional 9 days over concentrated sulfuric acid. They were cultured as described.

Calf Transmission Study. Attempts to transmit D. congolensis from infected to non-infected calves were conducted using 11 clinically normal calves. Six Shorthorns, 4 Herefords, and 1 black crossbred Jersey, ranging in weight from 300 to 450 pounds were used.

The clinically normal calves were examined for D. congolensis

by collecting skin scrapings, hair, and skin debris on the day of initial scarification. Microbiologic examination was performed as described in the reservoir study. Preparation of the skin sites consisted of shaving and scarifying an area 10 cm. X 15 cm. over the top of the shoulders. The depth was approximately 2 mm. This area was selected because it provided flies a long, unhampered feeding time.

Each calf was held 3 weeks. The scabs which had formed were collected every 5 to 7 days for microbiologic examination. At the time of scab removal, a new site adjacent to the previous scarification was shaved and scarified. This was done because it had been observed that repeated scarification on the same site did not result in good scab formation.

Organism Source. One severely infected calf[4] was used as the source of D. congolensis for a period of two weeks. Recovery of this calf was so rapid that by the end of the 2-week period, active lesions were difficult to demonstrate. The calf was used as an artificially infected animal for the remainder of the investigation. In addition, 2 artificially infected calves also served as sources of the organism. Areas on the backs of these calves were prepared similar to those of the experimental calves. They were also inoculated topically with 10 ml. of a nutrient broth culture as described in the pathogenesis study. The culture was examined for viability and purity on the day of animal inoculation. Scabs

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[4]Donated to KSU Veterinary Research Farm for experimental purposes by Ralph Johnson of Pomona, Kansas.

were collected every 5 to 7 days and examined microscopically by smearing on glass slides and staining with Giemsa stain.

At the time the scabs were removed an area of the skin adjacent to the previous scarification site was shaved, scarified, and infected.

Fly Exposure. Calves were held in heated stalls maintained at 80 F. The stalls were separated in the middle by two partitions 24 inches apart to prevent physical contact between the calves. One normal calf was placed on one side of the stall and one infected calf was placed on the other side of the stall. Propagating colonies of S. calcitrans were placed in two stalls with the calves. Five hundred S. calcitrans pupae[5] were obtained at 3-week intervals for use in a third stall.

Microbiology. The same methods were employed for both cultural and microscopic examination of the scab material as was described in the pathogenesis studies.

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[5] Stomoxys calcitrans. Provided by Dr. Robert Harris, USDA, ARS, Entomology Research Division, Livestock Insects Investigation, Kerrville, Texas.

RESULTS

Animal Reservoir Studies

External Examination. Dermatophilus congolensis was not isolated from cutaneous material collected from the skin of 9 calves originating from farms where streptothricosis had been diagnosed nor from skin of 15 calves originating from farms where cutaneous streptothricosis had not been diagnosed.

Blood Study. Dermatophilus congolensis was not isolated from blood samples obtained from 23 cattle from a known infected area.

Pathogenesis Studies

Rabbit Experiment. Rabbits 1, 2, and 7, which had been inoculated intradermally with a culture of D. congolensis, developed abscesses at the inoculation sites. These abscesses consisted of a thick, tenacious, cream colored, purulent material. Dermatophilus congolensis was isolated from these abscesses. The abscesses healed in 10 to 14 days. Scabs formed at the scarification sites were negative for the organism. Rabbits 3, 4, and 8 which had been inoculated subcutaneously, did not develop abscesses and D. congolensis was not isolated from the scarification site.

Rabbits 5, 6, 9, 17, and 18 were inoculated intravenously and the skin scarified simultaneously. Dermatophilus congolensis was isolated from ground scab material from rabbits 6, 9, and 18. The colonies were

first detected by a circle of beta hemolysis 1 to 2 mm. in diameter that appeared 36 to 48 hours after the plates were inoculated. Close examination revealed very small colonies in the center of the zone of hemolysis. The agar was slightly indented around the minute colonies and within 48 to 72 hours incubation time the colonies became readily visible. These colonies were yellow-orange in color and verrucose in appearance. The zone of hemolysis surrounding the colonies extended 1 to 2 mm. from the colony edge. This zone was greater than that commonly observed surrounding colonies produced upon routine transfer of D. congolensis onto 10% sheep blood agar plates.

Cultural and microscopic examination of scab material collected from rabbits 5 and 17 five days following scarification and intravenous inoculation failed to reveal the organism.

At the time the scab was removed from rabbit 17, a generalized swelling of both ears was observed and there was some degree of scab formation, particularly at the tip of the ears. This was attributed to trauma resulting from intravenous inoculation and application of xylene. Ten days after the removal of scab material (15 days after inoculation and scarification), 3 wart-like lesions appeared in the short hair growth at the scarification site. Several lesions were also found on the ears. These resembled the "paint brush" lesions frequently observed on the backs of affected cattle. Removal of lesions from the scarification site resulted in hemorrhage; removal of "paint brush" lesions from the ears revealed

raised, reddened, granulararеas on the attachment surfaces. Microbiologic examination of scab smears and ground scab material from the back and ears revealed D. congolensis.

Seven rabbits (10, 11, 12, 13, 14, 15, 16) of Group III were inoculated at one time and scarified at various time periods. Dermatophilus congolensis was not isolated from scab material. These rabbits and rabbits 17 and 18 were examined prior to intravenous inoculation and did not reveal D. congolensis.

Histopathology. Histopathologic examination of skin biopsies from rabbits of Groups III and IV did not reveal D. congolensis. Superficial necrosis of the skin was noted in all sections.

Necropsy Data. Rabbit 5 did not have any gross lesions. Rabbit 6 had an abscess 3 mm. in diameter in the liver together with numerous pale infarcts in both kidneys. Cultural and histopathologic examinations of liver and kidney were negative.

Rabbits 10 and 18 did not have gross lesions. Rabbit 11 had a secondary infection at the skin biopsy site. No internal gross lesions were found.

Calf Experiment. Dermatophilus congolensis was not isolated from scabs collected prior to intravenous inoculation. Scabs removed 5 days following rescarification and inoculation did not reveal the organism.

Temperature Readings and Blood Studies. Rectal temperatures and blood studies on 3 calves (335, 369, 371) were in the normal range for

cattle. (Tables 1, 2, and 3).

Transmission Studies

Fly Examination. Attempts to isolate D. congolensis from S. calcitrans were unsuccessful. Bacterial and fungal contaminants overgrew the plates within 24 to 48 hours incubation time even though Polymyxin B Sulfate was used as an inhibitor. The sulfuric acid drying method also failed to inhibit contaminants.

Fly Transmission of Dermatophilus congolensis to Calves. Dermatophilus congolensis was not isolated from the 11 calves prior to transmission studies. Dermatophilus congolensis was isolated from 1 calf 5 days following first scarification and fly exposure to an infected calf. The organism was demonstrated both by the scab smear and scab culture technique. The S. calcitrans population was estimated at near 200 which was considerably lower than the number of flies to which the other 10 calves were exposed.

The 2 stalls in which fly colonies were propagated contained 1,000 to 2,000 flies throughout the experiment. The fly population of the third stall into which pupae were introduced numbered from 200 to 400 flies. During one three-week period only, the third stall contained approximately 1000 flies.

TABLE 1. Temperature Readings and Hemogram Results on Calf No. 335 (Pathogenesis Studies)

Days Prior to Inoc.	Temp. (F)	W. B. C. (cells/cu.mm.	HB. (gms.)	P. C. V. (vol.%)	Bas. (%)	Eos. (%)	Myelo. (%)	Band. (%)	Neut. (%)	Lymph. (%)	Mono. (%)
11	104.6	9650	10.2	31.0	0	0	0	0	28	72	0
6	102.0	8958	11.0	28.0	0	0	0	0	20	80	0
3	—	8412	10.2	31.0	0	1	0	0	31	66	2
Day of Inoc.	103.6	7149	10.4	32.0	0	1	0	0	28	70	1
Days after Inoc.											
1	103.0	8403	10.4	30.0	0	2	0	0	32	66	0
2	103.0	7609	8.8	26.0	0	0	0	1	31	67	1
4	103.2	8293	10.2	30.0	0	0	0	0	32	68	0

TABLE 2. Temperature Readings and Hemogram Results on Calf No. 369 (Pathogenesis Studies)

Days Prior to Inoc.	Temp. (F)	W. B. C. (cells/cu.mm.)	HB. (gms.)	P. C. V. (vol.%)	Bas. (%)	Eos. (%)	Myelo. (%)	Band. (%)	Neut. (%)	Lymph. (%)	Mono. (%)
11	102.8	10520	12.7	36.0	0	0	0	0	17	83	0
6	101.8	9668	14.6	37.0	0	6	0	0	24	70	0
3	—	7705	11.4	36.0	0	4	0	0	40	55	1
Day of Inoc.	102.2	7589	10.8	32.0	0	2	0	0	36	62	0
Days after Inoc.											
1	102.0	7574	12.4	36.0	0	0	0	0	44	55	1
2	101.8	6708	10.8	32.0	0	2	0	0	31	65	2
4	102.0	7163	11.6	32.0	0	2	0	0	35	61	2

TABLE 3. Temperature Readings and Hemogram Results on Calf No. 371 (Pathogenesis Studies)

Days Prior to Inoc.	Temp. (F)	W. B. C. (cells/cu.mm.)	HB. (gms.)	P. C. V. (vol.%)	Bas. (%)	Eos. (%)	Myelo. (%)	Band. (%)	Neut. (%)	Lymph. (%)	Mono. (%)
11	104.5	14815	12.0	33.0	0	0	0	0	45	54	1
6	102.2	9271	17.8	38.0	0	1	0	0	16	82	1
3	—	9293	11.4	36.0	0	2	0	0	35	63	0
Day of Inoc.	103.4	7855	11.0	32.0	0	0	0	0	23	74	3
Days after Inoc.											
1	103.4	8573	13.4	35.0	0	3	0	0	23	63	1
2	103.4	8288	11.4	35.0	0	0	0	0	15	83	2
4	103.2	8354	11.4	36.0	0	1	0	0	17	80	2

DISCUSSION

Reservoir studies were conducted which indicated that D. congolensis was probably not a saprophyte of bovine skin or blood.

Very little information pertaining to examination of normal animals was found in the literature although several investigators suggested that the organism was a saprophyte existing on the hair and skin.

In a survey of 12 healthy sheep, Mason and Bakker (1934) were unsuccessful in isolating the organism of mycotic dermatitis. Roberts (1963b) considered actively infected sheep to serve as the source of outbreaks.

One reason more investigations of healthy animals have not been conducted is that examination of normal animals would be very difficult because there are no known selective media for organism isolation (Macadam, 1962). Polymyxin B Sulfate was employed as a partial inhibitor of contaminants in the present investigation and gave satisfactory results.

Better material than hair, skin debris, and skin scrapings for microbiologic examination would improve the external examination of clinically normal animals. One method that might be employed would be to apply the ground hair and skin scrapings directly to shaven, scarified areas on the animal from which they were removed. The scabs could then be examined by the scab smear and scab culture methods described in this investigation. To further justify this procedure, it has been

found that by grinding contaminated, infected scab material and applying the ground material to scarified areas on clinically normal animals, almost pure cultures of D. congolensis could be obtained from the scabs. Additional reservoir studies of clinically normal animals as well as examination of soil from premises where infected herds are identified provide additional areas for study.

Occurrence of the disease in Kansas herds has predominantly been detected in suckling calves. Roberts and Vallely (1962) noted a similar occurrence in England and suggested the infection was introduced during the drying stage of the calf after birth. This would seem to implicate soil or vegetation as the reservoir for the organism. Another possible reservoir of the organism is the oral cavity. Actinomyces israeli, of the same Order as D. congolensis, has been isolated from the mouths of humans showing no evidence of infection (Rosebury et al., 1944).

Studies of pathogenesis showed that by intravenous inoculation, organisms could be recovered at the scarification sites if the scarification and inoculation was made at the same time. Dermatophilus congolensis was not isolated from scarification sites when such scarification was made 2, 4, and 6 days following inoculation. These data indicated that the appearance of the organism was a result of increased blood supply and hemorrhage at the scarification sites, thus serving as an internal application of organisms to the injured area. The fact that the organism did not survive in the circulatory system would indicate

that this route of infection was of little significance under field conditions.

Other investigators have conducted pathogenicity studies but did not incorporate scarification as a portion of their investigations. Chodnik (1956) inoculated rabbits and guinea pigs intraperitoneally, intravenously, and subcutaneously. His findings were negative except for the subcutaneous inoculation which developed an abscess that ruptured and healed rapidly. Mémery and Mémery (1962) inoculated 12 rabbits, 2 adult goats, a suckling goat, and a calf with massive intravenous doses of the organism. They reported the calf and goats had only a transient rise in temperature. Two of 12 rabbits developed skin lesions and cachexia similar to that seen in cattle.

In this investigation, positive results in rabbits following intravenous inoculation, and negative results in calves following intravenous inoculation could not be explained. The dosage for calves may have been too low or the rabbit may be more susceptible. It has been shown experimentally that papillomatosis in the rabbit can be produced at a scarification site following intravenous injection of the causative virus, (Stewart et al., 1959).

The development of streptothricosis in rabbit 17, fifteen days following intravenous injection, probably resulted from additional factors other than the intravenous inoculation. Trauma was produced at the time of intravenous inoculation. This, combined with the xylene application, could have provided factors necessary for the growth and multiplication

of the organism. Circumscribed lesions were present along the larger ear veins where the most trauma was produced. At the tip of the ears where blood vessel anastomosis is greatest, the lesions were more confluent. The reason for development of lesions at the ear tips where trauma was absent could possibly be explained two ways: (1) the irritating action of the xylene plus external contamination of the organism or (2) the irritating action of the xylene causing localization of the circulating organisms in the blood vessel anastomoses.

The lesions on the back of this rabbit developed after removal of scabs for microbiologic examination. These could have resulted from contamination by the ear tips or by the active lesions of the ears producing a systemic infection which localized at the healing scarification site.

Further studies could be conducted using irritants as a source of skin injury to help clarify the possible role of skin irritation and injury in the epidemiology of cutaneous streptothricosis.

Histopathologic examination of 9 biopsies removed from rabbits of Groups III and IV were made to determine if intravenous inoculation would result in appearance of the organism in the skin, in the scabs, or on the surface of skin. Albiston (1933) found mycelia in the hair follicles and Zlotnik (1955) described the pathologic process as beginning with the penetration of the mycelia into hair follicles with multiplication of the organism in the external root sheath and hair papilla,

leaving the hair shaft intact. Weide (1964) demonstrated mycelia in hair follicles from biopsies of skin taken from Holstein-Friesian cattle infected with streptothricosis.

Rabbit skin biopsies taken in this investigation consisted only of skin from which the scabs had been removed for cultural and microscopic examination. Dermatophilus congolensis was not observed in these biopsies.

Transmission studies showed that it was possible for S. calcitrans to serve as a vector of D. congolensis. However, in this experiment, transmission was accomplished in only 1 of 11 trials.

Stomoxys calcitrans are known for their free-flying habits. To take advantage of this characteristic both sides of the partitioned stalls were equally lighted so the flies would move freely back and forth between the calves. Numerous flies were observed feeding on the scarified areas of both calves in each stall especially after emergence from the pupal stage. In addition to feeding on the scarified areas, flies were noted on the legs, particularly from the knees and hocks to the coronary bands.

Macadam (1964), in his experiment with S. calcitrans, used a single animal with infected and non-infected scarified or shaved areas. The problem of results obtained in such a manner are: (1) the animals probably could contact both inoculated and uninoculated areas with the head, tail, or feet in attempting to drive off the flies and consequently

cross contamination could occur, or (2) by rubbing non-infected scarified areas on posts, feeders, ropes, gates, etc. which had been contaminated with organisms from initial infected lesions.

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RESERVOIR, PATHOGENESIS, AND TRANSMISSION STUDIES OF *DERMATOPHILUS CONGOLENSIS* VAN SACEGHEM

by

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Literature pertaining to the reservoir, pathogenesis, and transmission of the causative agent of cutaneous streptothricosis is both confusing and contradictory. This investigation was undertaken in an attempt to clarify some features of the reservoir, pathogenesis, and transmission of Dermatophilus congolensis Van Saceghem.

The culture of D. congolensis used in the investigation was isolated from scab material removed from an animal infected with streptothricosis. Ten percent sheep blood agar containing 1000 units of Polymyxin B Sulfate per ml. as an inhibitor was utilized throughout the investigation for isolation of the organism from potentially contaminated material. Identification of the pathogen was based on microscopic morphology and macroscopic appearance of the colony.

Reservoir studies of the clinically normal bovine were conducted on animals originating from infected and non-infected herds. External examination of 24 calves and blood culture examination of 23 cattle of mixed ages and breeds did not reveal D. congolensis.

Pathogenesis studies were conducted using 18 rabbits and 8 calves. The skin was scarified at one site and the animals were inoculated at another site. Intradermal inoculation of 3 rabbits resulted in abscess formation from which D. congolensis was isolated. The abscesses healed in 10 to 14 days. Subcutaneous inoculation of 3 rabbits did not result in abscess formation at the inoculation site and D. congolensis was not isolated from the scabs formed at the scarification site. Intravenous

inoculation of 7 rabbits which were scarified the same day resulted in the presence of D. congolensis in the scabs formed at the scarification site on 3 rabbits. One of the remaining 4 rabbits developed an active infection of cutaneous streptothricosis 15 days following scarification and inoculation. This was attributed to trauma and chemical irritation (xylene) of the skin at the time of inoculation.

The skin of 5 rabbits was scarified at varying times following intravenous injection. No gross lesions were observed and no isolations of D. congolensis were made from these animals.

Histopathologic examination of skin removed from the scarification site of 9 rabbits injected intravenously did not reveal D. congolensis in the skin.

Necropsy of 5 rabbits inoculated intravenously with 3/4 ml. of a 5-day nutrient-broth culture of D. congolensis was performed. Numerous pale infarcts in both kidneys and a liver abscess 3 mm. in diameter were observed in one rabbit. The organism could not be demonstrated by histopathologic examination or by culture. The remaining 4 rabbits exhibited no gross lesions. The 8 calves inoculated intravenously with 10 ml. of a 5-day nutrient-broth culture of D. congolensis failed to reveal the organism at the scarification site.

Transmission studies were conducted using Stomoxys calcitrans as the vector. Dermatophilus congolensis was not isolated from flies fed on defibrinated sheep blood inoculated with 5-day broth cultures of

D. congolensis.

One artifically infected calf and one normal calf were placed in each of 3 stalls partitioned to prevent physical contact. The temperature was maintained at 80 F. Fly colonies were propagated in two of the three stalls. Five hundred S. calcitrans pupae were added to the third stall every three weeks. The fly population was estimated to be 1000 to 2000 in each of the propagation stalls throughout most of the experiment. Dermatophilus congolensis was isolated from the scabs removed from 1 of 11 calves which did not show presence of D. congolensis prior to fly exposure.

Further reservoir studies should be conducted with emphasis placed on soil examination. The use of various types of skin irritants other than mechanical agents would be of benefit in future pathogenesis and transmission studies.