Isolation of *Actinobacillus seminis* from Ovine Epididymitis

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SUMMARY

A microorganism isolated from the semen of a ram with an epididymal infection was identical morphologically and culturally to *Actinobacillus seminis*, which has been reported only in Australia. Biochemical reactions were identical, except that the Texas isolate did not produce acid in trehalose. A diagnostic rise in serum antibody titer was demonstrated with serum obtained from rams experimentally infected with the Texas isolate and antigen prepared from the Australian isolate.

Epididymitis has been observed in rams for a number of years. In Australia, Simmons and Hall [4] isolated an organism from infected epididymides of rams and were able to transmit the condition. They described the organism as being "brucella-like" but were unable to classify it. Buddle [2] concluded that a new specific name should be made available for this organism and he proposed the name *Brucella ovis*. This disease was first reported by McGowan and Shultz [4] as occurring in rams in the United States.

An organism similar to *Br. ovis* and producing lesions in the epididymis was reported by Baynes and Simmons [1] in Australia. This organism, which appeared to be a new species, was identified as an Actinobacillus. Since it had been isolated from semen, the specific name *A. seminis* was suggested. These workers stated it was very unlikely that the condition would be differentiated from *Br. ovis* infection by clinical examination alone. Therefore, laboratory procedures are necessary to determine the cause of epididymitis in each affected animal.

Case History

On Jan. 18, 1962, yearling Rambouillet ram 701 was found to have enlarged testicles. This ram was 1 of a group of 4 which had been brought to the Texas Agricultural Experiment Station, Substation No. 14, Sonora, 4 months previously by a private owner for the purpose of progeny testing. This group of rams had been placed in a pen with several other groups of rams, making a total of 46. All groups were of different geographic origins. Ram 701 was the only one in the pen with signs of this condition, although he was allowed to remain in contact with the other rams in the pen until the progeny test was completed 2 months later. The testicles remained enlarged for several weeks and then gradually decreased to normal size; however, epididymal lesions were palpable as long as the ram was observed. The owner would not permit us to kill the ram for further examination.

Materials and Methods

Isolation of the Causative Organism from the Semen of a Naturally Infected Ram.—Semen collected in sterile test tubes from ram 701 by means of an electroejaculator was streaked on 10% sheep blood agar plates. The inoculated plates were incubated at 37 C., aerobically and in 10% CO_2. Selected colonies were inoculated into brain-heart infusion broth containing 10% unheated calf serum.

Identification of the Isolate.—Smears made from selected colonies on the blood agar plates were heat-fixed and stained with Gram's stain. Duplicate smears were stained by the modified Ziel-Nielson staining method. Hanging-drop mounts were prepared from an 18-hour broth culture of this organism. The following substances were prepared in a 0.5% concentration in phenol red broth base: maltose, dextrose, lactose, sucrose, salicin, trehalose, inulin, sorbitol, arabinose, xylose, glycerol, fructose, galactose, dextrin, mannose, mannitol, rhamnose, dulcitol, aesculin, and inositol. These mediums were inoculated with the organism which had been grown in brain-heart infusion broth for 4 days. Later, similar inoculations were made with the organisms after it had been maintained on an artificial medium for 8 months.

Transmission of Epididymitis Using Semen from a Naturally Infected Ram.—One milliliter of semen from ram 701 was injected into the left

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testis of Rambouillet ram 702. This ram was then examined periodically for signs of epididymitis. Semen was obtained by electroejaculation.

Transmission of Epididymitis Using Broth Cultures of Isolate Obtained from Ram 701.—Two Rambouillet rams, 750 and 751, were obtained from the flock at the substation. Both had normal testicles and produced normal semen. One milliliter of a 10% calf serum brain-heart infusion broth culture representing the 5th serial passage of this isolate on artificial medium was injected into the tail of the right epididymis of ram 750. One milliliter of the identical culture was injected into the right testis of ram 751. Semen was obtained from both rams by electroejaculation and cultured on 10% sheep blood agar plates at varying intervals. Blood serum was obtained before inoculation and at 30 days after inoculation. Both rams were castrated 27 days after inoculation, and specimens were obtained for cultural and pathologic examination.*

Serology.—Actinobacillus seminis antigen and known positive antiserum for this organism were obtained.** A direct complement-fixation test was done with the known antigen, known positive serum, serum obtained from rams 750 and 751 before inoculation with the Texas isolate, and serum obtained from the same rams 30 days after inoculation.

Results

Isolation of the Causative Organism from the Semen of a Naturally Infected Ram.—Microscopic examination of semen obtained from ram 701 revealed the absence of spermatozoa and an abundance of neutrophils. Small, pin-point nonhemolytic colonies were found on the 10% sheep blood agar plates after 24 hours' incubation under both aerobic and 10% CO_2 conditions. These colonies grew under aerobic conditions, but they developed more rapidly under increased CO_2. The colonies were round, convex, with an entire border, and grayish white. After 3 or 4 days of incubation, the larger colonies were 3 to 4 mm. in diameter and umbonate. Organisms taken from these colonies were gram-negative, pleomorphic rods ranging from coccobacillus forms to long rods (1 μ to 5 or 6 μ). The organisms were non-acid-fast when subjected to the modified Ziel-Nielson staining method and were nonmotile. This organism was isolated from ram 701 at 30 days and 60 days after primary isolation.

Identification of the Isolate.—All fermentation reactions were negative at 21 days. At 28 days of incubation, acid reactions were present in mannitol, fructose, and arabinose. After this organism had been grown for 8 months on artificial mediums, the time required to produce an acid reaction in these same carbohydrates was reduced to 4 days. Litmus milk was not changed. To support growth of the freshly isolated organisms, 10% serum was required. After the organism became adapted to artificial mediums, serum did enhance growth but was not essential. After 24 hours' incubation in serum brain heart infusion broth, growth was barely discernible. After 48 hours' incubation, granular particles formed and settled to the sides of the tube.

Transmission of Epididymitis Using Semen from a Naturally Infected Ram.—Twenty-four hours after inoculation the left testicle was slightly enlarged, whereas 4 days after inoculation it was twice its original size. Forty-eight days after inoculation both testicles had atrophied, and the left testicle was one-half the size of the right testicle. At this time microscopic examination of the semen revealed only a few dead spermatozoa and a few neutrophils.

Ninety-eight days after inoculation changes were not observed in the testicles since the previous examination. Semen examination revealed only a few live spermatozoa in masses of dead spermatozoa.

Transmission of Epididymitis Using Broth Cultures of Isolate Obtained from a Naturally Infected Ram.—Forty-eight hours after inoculation the right epididymis of ram 750 was more firm than the left. Seventy-two hours after inoculation the right epididymis was noticeably enlarged and very firm. Microscopic examination of the semen revealed neutrophils and a below-normal number of spermatozoa. Twenty-one days after inoculation the causative organism was isolated from the semen. At this time the density of the semen and motility of the spermatozoa were judged to be good. Twenty-seven days after inoculation the testicles were removed and examined. Adhesions of the tunica vaginalis to the right epididymis in the groove between the tail of the epididymis and the right testis were observed. The adhesions had extended to the body of the epididymis. There was an abscess,

* Pathologic examination performed by Dr. Dee O. N. Taylor, associate professor, Department of Pathology, School of Veterinary Medicine, Texas A & M University, College Station.

** Obtained from Mr. G. C. Simmons, Animal Research Institute, Yeerongpilly, Brisbane, Queensland, Australia.

2.5 cm. in diameter, surrounded by a capsule in the tail of the right epididymis. The causative organism was isolated from the abscess contents. The left testicle appeared to be normal on gross examination. Microscopic examination revealed aspermatogenic tubules and edema between the seminiferous tubules. Many of the tubules were lined with degenerating cells and were filled with cellular debris. Part of the epididymis was involved in a granulamatous complex of macrophages and giant cells. Many spermatozoa had been phagocytized by these cells. Considerable fibrous connective tissue surrounded the lesion. Occasional segments of the ductus epididymidis peripheral to the main lesion were surrounded by lymphocytes, plasma cells, and macrophages. Occasional macrophages were seen in the duct lumen.

Forty-eight hours after inoculation the left testicle of ram 751 was only slightly enlarged. Seventy-two hours after inoculation the testicle and semen appeared normal, and semen cultures were negative.

Twelve days after inoculation the testicles and semen appeared normal, and semen cultures were negative.

Twenty-one days after inoculation the testicles and semen appeared normal, but the causative organism was isolated from the semen.

Twenty-seven days after inoculation the testicles were examined grossly and appeared normal. Material obtained from the testicles did not yield the causative organism. Microscopic examination revealed an increase of connective tissue around the epididymis. There was edema between the convolutions of the ductus epididymidis. Some vessels in these areas were surrounded by lymphocytes and macrophages. Mild infiltration of lymphocytes was observed in the tunica vaginalis propria. Some seminiferous tubules had accumulations of proteinaceous fluids in them.

Serology.—Known positive serum produced a 4+ reaction at a 1:32 dilution. Serum obtained from ram 750 before inoculation was negative.

Serum obtained from ram 750, 30 days after inoculation, produced a 4+ reaction in tube 4 (dilution 1:16). Serum obtained from ram 751 before inoculation was negative. Serum obtained from the same ram 30 days after inoculation produced a 4+ reaction in tube 3 (dilution 1:8). All controls were satisfactory.

Discussion

The organism isolated from the naturally infected ram 701 was identical in cell structure, colony characteristics, modified acid-fast staining reaction, and serum requirements for primary isolation to *A. seminis,* which was isolated by Simmons in Australia. Biochemical characteristics were identical during the first 3 weeks of incubation. At 4 weeks, Simmons reported that both Australian strains produced slight acid in arabinose, fructose, and trehalose, and 1 strain produced acid from mannitol. The Texas isolate produced acid in fructose, mannitol, and arabinose after 4 weeks' incubation. Trehalose was not fermented by the Texas isolate. Both organisms are identical serologically, because a rise in titer was observed in the serum from experimentally infected rams 750 and 751 to the antigen supplied by Australian workers.

Until now this organism has only been reported in Australia, where the first isolation was made from 3 naturally infected rams. *Actinobacillus seminis* and *Br. ovis* infections produce similar lesions in rams and are similar in appearance and biochemical reactions. It is possible that *A. seminis* infections have been confused with infections caused by *Br. ovis.* This may be one reason that negative serologic results with *Br. ovis* antigen have been obtained with serum from rams with palpable lesions in the epididymides.

The manner and route of infection has not been determined. The ram from which this organism was isolated had never been bred and was in contact with other rams which had never been bred. It is surprising that none of the other rams in this pen developed epididymitis, although the infected one was allowed to remain in contact with them for 2 months. Infected ram 701 was certainly a potential spreader of the infection, because *A. seminis* organisms could be isolated from the semen for at least 2 months after the infection was first discovered. Also, the organism was recovered from the semen of 2 rams (750 and 751) 21 days after inoculation. Quality of semen from both rams was judged satisfactory. Ram 751 had no visible signs of infection. Thus, it appears wise to cull and eliminate from the flock any rams known to have had epididymitis, even though the semen quality is acceptable.

References

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SUMMARIO IN INTERLINGUA

Isolation de Actinobacillus seminis ab Epididymitis Ovin

Un micro-organismo isolate ab le semine de un ove mascule con infection epididymidal esseva morphologicamente e culturalmente identic con *Actinobacillus seminis* le qual, usque nunc, ha essite reportate solmente in Australia. Le reactiones biochimic esseva identic, excepte que le isolato de Texas non produceva acido in trehalosa. Un augmento diagnostic in le titro de anticorpore seral esseva demonstrate con sero obtenite ab oves mascule que habeva essite inficite experimentalmente con le isolato de Texas e antigeno preparate ab le isolato de Australia.