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Bacteriologic Observations on Periodic Ophthalmia in Horses.

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Through the cooperation of Dr. F. Park Lewis of Buffalo, N. Y., I have had opportunity to study the bacteriology of periodic ophthalmia in horses. This disease is said to be the greatest single menace to the horse industry, the world over.

By means of animal inoculation or cultural methods, an organism having specific affinity for the eye has been isolated from the lacrimal sac in 19 of 40 cultures made in 16 affected animals. It has been isolated at least once in 12 of the affected animals, twice in 4 and 3 times in two. The inflammation of the eyes of the 4 animals that yielded negative results had either entirely disappeared or was receding at the time the swabbings were made. It has been isolated from the lacrimal sac in only 2 of 28 normal horses of the herd affected and in no instance of 23 horses cultured remote from the outbreak. It has been isolated from the mucous membrane of the nose in 2 of 6 affected animals, from a sample of water containing yellowish pieces of scum or moss from the shaded side of the tub outdoors, to which the herd had access, and from the water contained in a tub within the stable supplying the drinking water for 10 of the affected animals. Similar cultures from 13 samples of water obtained from brooks, pools, hoof prints, and wagon tracks in the pasture failed to yield the bacillus in a single instance. It was isolated from one specimen of the oats and pea hay which was fed last winter when the epidemic began. It was not obtained in cultures from the feces of 3 nor from the winter coat of hair of one of the affected animals. It has been isolated from the fluid aspirated from the anterior chamber in 4 of 6 acutely affected eyes, and has been isolated in pure culture or in association chiefly with a large diplococcus, from the fluid in the anterior chamber, and emulsions of the exudate covering the iris, the iris, uveal tract, and limbus of an eye of a horse which was removed under chloroform anesthesia for study, early in the third and worse attack.

The suspected organism is a medium-sized Gram negative, motile rod with somewhat rounded ends. It grows rather slowly aerobically on the common culture mediums. On plain or glucose-agar and plain horse blood-agar it produced medium-sized glisten-

ing, translucent, non-adherent, yellow to orange colonies with smooth margins, and on potato, a spreading confluent yellow growth. The yellow pigment formation is not as helpful for the isolation of the organism from mixed cultures as it would seem, for in some strains it does not develop until after 48 to 72 hours' incubation and may not appear at all where growth is heavy or where available oxygen is limited. In plain and dextrose broth it produces a moderate, diffuse clouding, most dense in the upper layer and in older cultures, a yellowish pellicle is formed. The pigment remains within the organism; flourescence has not been observed. It produces no change in litmus milk, liquifies gelatin slowly, does not digest Loeffler's blood serum, does not form indol, ferments sugars slightly without gas production, and reduces nitrates to nitrites. Its size and grouping varies considerably, depending upon whether grown in liquid or solid mediums. It usually stains diffusely although polar staining and clear areas sometimes resembling spores occur in a few individual bacilli, especially in old cultures. On agar the organism is quite uniform in size, ranging from 0.5 by 1.2 to 0.7 by 2.5 microns and thread formation is usually absent. In liquid cultures the length of individual rods varies considerably and short threads are sometimes seen. Flagella have not yet been demonstrated. Only a small proportion of the organisms are found actively motile, even in young cultures. It is not acid fast. Young cultures are readily killed by heat and antiseptics, grow rather slowly at 35 to 37° C., and somewhat more quickly at room temperature, the optimal temperature for growth ranges between 25 to 30° C. According to Bergey's classification, the organism belongs to the genus *Flavobacterium,* but it does not conform in all particulars to any of the numerous varieties described as peculiar to this genus.

Great difficulty has been experienced in isolating and maintaining the organism in pure culture. It has happened repeatedly that fishings from well isolated single colonies on platings containing seemingly only characteristic colonies in which smears reveal typical Gram negative bacilli only, and in which young cultures proved to be pure, that Gram positive or negative large or moderate sized cocci, diplococci or diphtheroid bacilli were found in the same culture after prolonged incubation. This has been a puzzling problem and at present it is uncertain whether we are dealing with inadvertent mixtures or mutations. The various forms found in these cultures, especially the large Gram positive or negative diplococcus, resemble very closely the organisms isolated commonly with the bacillus from swabbings of the lacrimal sac and from material obtained from within affected eyes.

When injected in relatively large doses, the organism is virulent for the rabbit, guinea pig, white rat, and the horse. The symptoms and death in each of these species, however, is due apparently chiefly to the presence of toxic material preformed in the broth or to that liberated from the organisms injected. It disappears quickly from the blood after injection in the animal that survives, and is never found in large numbers in blood and other tissues in animals that succumb. It tends to localize in the anterior structures of the eye when injected intravenously.

The reaction in the animals injected in proper dosage is very characteristic, especially in the rabbit. Soon after injection in this animal of 2 to 5 cc. of a broth culture or filtrate per 1000 gm. of body weight, the respirations become increased, weakness becomes manifest, often associated with fine tremors of muscles, especially on exertion, and coincidentally there appears congestion of the vessels of the palpbral and ocular conjunctiva, of the iris, and especially of circumcorneal vessels, and a variable degree of lacrimation. Similar, although milder, symptoms have followed intravenous injections of as little as 0.1 cc. If the animal recovers, the findings in the eye disappear. In one instance turbidity of the fluid in the anterior chamber and other evidence of infection has developed from intravenous injection. Placing the organism in the lacrimal sac with and without injuring the cornea has thus far not resulted in intrinsic infection of the eye. However, a condition strikingly like the specific disease has been produced in several horses and a series of rabbits, by injecting into the anterior chamber, a small amount of pure cultures and filtrates of old cultures. The disease has been induced in both eyes of one horse, by injecting the fluid from the anterior chamber into one, and the emulsion in sodium chloride solution of the membranous exudate covering the anterior surface of the iris into the other. The eye from which this material was obtained was removed on the fourth day of the third typical spontaneous attack. Marked clouding, especially in the lower half of the anterior chamber occurred within 24 hours. The picture in both eyes 10 days since injection is thus far typical of periodic ophthalmia. The bacillus was isolated from the material injected and from the fluid aspirated from the eye receiving the emulsion. Both of these strains have produced the characteristic reaction on intravenous injection in rabbits. Injection of other organisms also isolated have shown little or no affinity for the eye and control injections of sterile broth are without effect. Filtrates of old cultures have been found extremely toxic, especially for the horse. Small amounts introduced into the anterior chamber cause marked cloud-

ing of the fluid, circumcorneal congestion, edema of conjunctiva, and lacrimation lasting for a number of days.

It has been found that intravenous or subcutaneous injection of filtrates of exhaust cultures render the eyes of rabbits and horses refractory to the effects of intraocular injections of the organism and filtrates, and in preliminary observations appear to lessen the inflammatory process in the eyes of affected horses. The filtrates are especially toxic for the horse. Two cc. injected intracutaneously and 2 cc. subcutaneously sometimes produces even in adult animals within from 1 to 2 hours, severe chilling, trembling of muscles, increased respiration and general illness in which the animals lie down and not infrequently, manifest unmistakable circumcorneal congestion. The eyes of rabbits that have fully recovered following injection of the living organism or toxic filtrate and the eyes of horses that have fully recovered from the spontaneous disease, appear more refractory to injection of the filtrates than the eyes of normal animals, whereas the eyes in both these species that still show inflammation, are more susceptible than those of normal animals. The skin of the rabbit, guinea pig, goat, monkey, dog and white rat is highly immune to filtrates, even the undiluted filtrate giving little or no reaction, whereas the skin of the horse is extremely sensitive, dilutions as high as 1 to 1000 often giving decided reactions. Presumptive susceptibility tests with filtrates of this organism have been made in a series of normal horses and in affected animals. Great variations in reactions have been observed and a parallelism between the involvement of eye and degree of the skin reactions have been observed in some of the affected herd.

Rabbits and horses are now being immunized in the hope of developing an antitoxic curative serum. The fact that the organism appears to produce a specific toxin measurable by intracutaneous injection, supports the hope that an antitoxin of high titre may be expected and which may perhaps be standardized by measuring its neutralizing power by intradermal injections of appropriate mixtures of toxin and antitoxin.

This is an abstract.