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TRANSLATION NO. 192

DATE: July 1968

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Additional findings in connection with experiments with and examinations of animal listeriosis.

by K. Diele Vet-hygienic and Epizootic Inst. of Karl Marx Univ., Leipzig

Discourse No. 10, Listeriosis, a symposium at Giessen, Germany, 27-28 June 1957, pp 99-104. Supplement 1 to Zentralblatt für Veterinärmedizin, 1958. with 1 graph.

Since I am speaking to an assembly of experts in listeriosis, I should like to dispense with the presentation of tests already published. I believe to serve the main purpose of our symposium, the discussion, better by way of description of our present examinations, even though they have not all been concluded as yet. In the following, agglutination, allergic diagnostic, type distribution, secretion and resistance, as applied to listeriosis of domestic animals, shall be discussed.

a) Agglutination

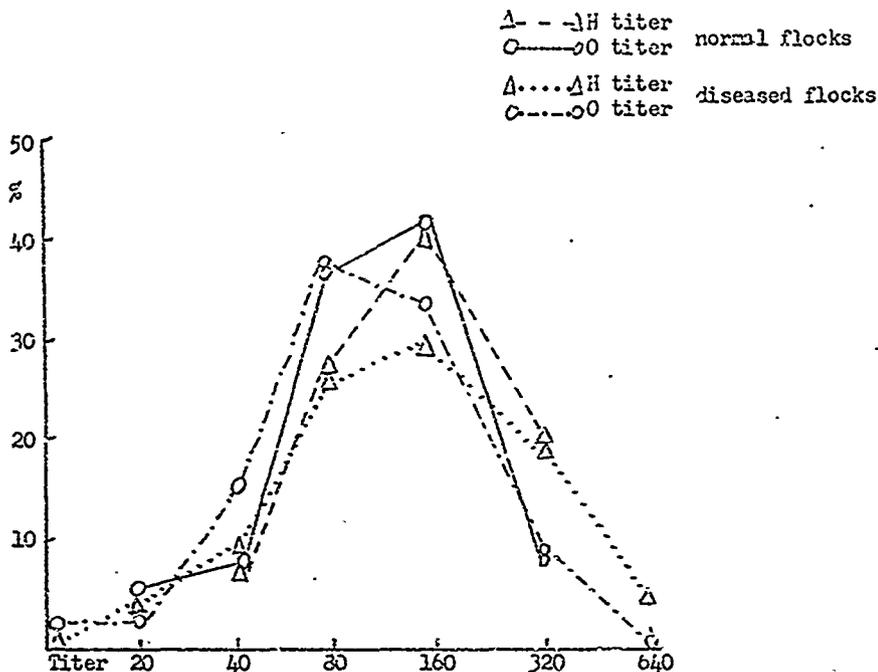
1. Sera of patients: For the past 2 years we have been regularly examining for listeriosis, sera from horses, swine and sheep in the clinic with uncertain, primarily central nervous symptoms. As antigen we used cultures in an ammonia solution: Na-amm. phosph. 1.5, prim. potassium phosph. 1.0, MgSO₄ 0.2 per 1,000 with 0.1% dextrose and 1% trypsin-peptone, pH 7.6, showing well readable results and distinct controls, but evidencing rather high sensitivity, i.e. they show a relatively high normal titer.

We have examined about 100 horses per year and considered a titer of 1:160 as still normal, experimentally. Of 4 animals with higher titers of H₆₄₀-O₃₂₀, three recovered without a sure diagnosis, one was sacrificed because of unspecific hepatic damage. These animals also showed blood changes such as leukocytosis, thickening of the blood and others. One pony with a titer of 640/52 was pregnant, but otherwise healthy.

About 50 sheep were examined yearly. Here the titers are usually between 80 and 160. Of 12 animals with proved cerebral listeriosis only one had a suspicious blood titer of H and O 160; all others were negative, i.e. up to or under 160. On the other hand several animals definitely afflicted with Dorna virus encephalitis revealed at times positive titers up to 320 next to low or negative values, so that nothing concerning the cause of disease could be deducted from the titers of animals delivered to the clinic with encephalitic manifestations.

A number of normal swine sera with titers of H₈₀-160, O₄₀-80 reacted similarly to those of sheep.

2. Flocks: Similarly to Linsert (8) we (9) compared agglutination results of two flocks afflicted with listeriosis with those of flocks free of listeriosis. The blood specimens so far taken from about 500 animals in the positive flocks and about 200 in the negative showed titers as depicted below.



Listeric titers in sheep flocks

If something were deducible from the titers concerning a latent infection of the flocks (which is very probable in view of the losses of 5% by clinical cerebral listeriosis), such should be reflected by a shift in the curves. Obviously this is not the case.

How can these findings be interpreted? We find, especially in young cricks that have been raised cleanly, completely negative titers or titers up to 1:10. We then conducted conjunctival and nasal infections of lambs and piglets resulting in serologically negative titers or titers of about 10, and have seen the titers rising to 80-160 without clinical signs of disease. A subsequent intravenous infection leads to further titer elevation. Swine could not withstand an intravenous infection, while lambs reacted hardly at all. In agreement with results already published by us (2) the temporary bacteriemia caused by intravenous infection led to titers of H640-1280, O80-320, which however sank to 80-320 H and 40-160 O within a few weeks, subsequently even lower.

It is then quite possible that titers of 40-160 may be attributed to previous contact of the animal with listeria or even to a pregnancy. We found listeria twice on the nasal mucous membrane of lambs previously infected at that spot, with titers of 40-80. On the other hand it is not impossible that in

connection with the low titers we are dealing with sympathetic agglutinations of antibody against partial antigens of other bacteria in Seeliger's sense or with unspecific flocculations. Staphylococci frequently occur in sheep, and hemolytic streptococci in young animals as well as adult horses, cattle and swine.

b) Allergic reactions

In view of the poor agreement between serologic and clinical findings we tried to achieve better results with cutaneous reactions with polysaccharide and endotoxin fractions. The extracts were made for us by the Research Institute for Vaccines, Dessau, from strains of the type I, IVa and IVb, to whom we again express our gratitude. The methods of manufacture have served well in the extraction of allergens from brucella, and we worked with listeria in concentrations which in experimental and spontaneous swine brucellosis resulted in typical, distinct redness with central necrosis and well visible or palpable swellings. Of these extracts type IVa precipitated with the homologous IVa serum in vitro, while in the complement fixation test none of the extracts showed an antigenic effect.

We prepared 2 pigs of 40-50 kg by giving one 3 X 5 ccm live culture of an ovine strain of type I at intervals of 3 weeks, while the other received the same amount of formal-inactivated culture intravenously. The first animal with a normal titer of H-0/10 reached H40-160/020-80 after preparation, the latter with normal titers of H10/010 reached H20-40/040-80, which points to a better effect of the flagellar antigen in live culture. Intravenous instillation of live culture in sucklings of 20-50 kg in two tests led to death following 36-hour long general septicemia, in spite of repeated preliminary immunization with vaccines of live culture subcutaneously or in the conjunctiva.

The extracts, of which the endotoxin contained 1.1% dry substance and the polysaccharide 0.5%, were injected intracutaneously at the flank, undiluted, 1:2 and 1:4 in doses of 0.2 ccm, 10 days after the last preparative injection. According to previous tests of serum allergy in swine the apex of reaction is reached 10-11 days after injection. An unprepared animal was treated identically. Due to its large, white cutaneous area the pig is well suited to such tests, according to our experience with serum allergy and brucellin.

The wheals on the pre-treated animal caused by the injection of antigen turn intensely red after a few minutes; the center remains pale when undiluted allergen is used. The redness was more bluish-red in connection with polysaccharide, more pink with endotoxin. The redness of the control animal was somewhat less intense and began to disappear after 5 hours. The redness of the pre-treated animal remained for 24 hours, without however increasing its diameter of about 1 cm. Swellings were not seen. In comparison to the impressive reactions to brucellosis, the wheals caused by listeric extracts were disappointing. Even the subcutaneous injection of 2 ccm of a mixture of both extracts led neither to swelling nor to a rise in temperature. The blood revealed a doubling or tripling of the total leukocyte count 3-4 days after injection and a rise in monocytes from 5-8 to 12-17% 24 hours after infection, if the animals were prepared with live culture.

c) Determination of type

The type determination of the strains isolated in our institute and at the veterinary health offices of Saxony-Anhalt did not reveal any fundamental news. We have about 90 strains on hand, of which 53 were isolated during the past 2 years.

32 strains were type I, and of these 26 came from sheep brains, 2 from human brains, 2 from ovine organs, 1 from a chicken, 1 from an infant.

19 strains were type IV, 4 of these IVb. Here we found 2 strains type IVa and 3 strains type IVb in sheep brains. Other IVa strains came from the organs of 1 sheep, 1 deer, 2 infants and 1 bovine fetus, and 8 were isolated by Eck from human brains in connection with cerebral listeriosis of a group (4); another of Eck's strains was type IVb. Type IVb is no rarer in animals than in man (6).

It was conspicuous that all of some 20 IVa strains which had been freshly isolated since the end of 1955, were rhamnose-negative. We utilized here a peptone water with 1% trypsin peptone from bulls' testes, 1% rhamnose and bromothymol blue as indicator. These rhamnose-negative strains usually are more hemolytic than others. Contrariwise, almost all IVb and older IVa strains are rhamnose-positive. There are also quite distinctly pronounced IVa and IVb variations and others which co-agglutinate with the heterologous variational serum. Finally, one finds occasionally different types in the same flock, sometimes even in the same sheep, so that no great epizootological significance should be ascribed to serologic type determination. The predominance of ovine brains among the test material is to be attributed to the diagnosis of viral encephalitis (Borna's disease) of sheep, important for preventive immunization.

Clinically, according to observations made at the medical veterinary clinic at Leipzig and own experience, we found the sheep ill with cerebral listeriosis to be febrile up to 42°C with corresponding pulse and respiratory frequencies. Gnashing of teeth, salivation, inappetence, facial paralysis, loss of pupillary reflexes, disturbed deglutition reflex are frequent symptoms. Circular movement with head held to one side, collapse and absolute ataxia occur frequently. Usually the younger sheep (up to 2 years of age) fell in the months from December to May, and the disease led to death $\frac{1}{2}$ to 3 wks after appearance of the symptoms. In Borna's disease, on the other hand, the depressive manifestations and manifold locomotive disturbances are more pronounced, and the duration of the disease at 1-1 $\frac{1}{2}$ weeks is distinctly longer. The symptoms in the area of the head are less impressive than in listeriosis. The blood picture and the cell count of the liquor may furnish good diagnostic pointers.

As mentioned above, we have never found positive agglutination titers of >160 in a sheep definitely ill with encephalitic listeriosis, as could be easily achieved by intravenous infection. The encephalitic form probably is not the effect of bacteriemia which had occurred shortly before. The Borna virus frequently occurring in Saxony also should not play a role in pathogenesis, since it is unknown in Mecklenburg, for example.

d) Epizootological examinations

1. Secretion. One can fundamentally count on finding occasional listeria as incidental findings or as secondary disease germs in all secretions and excretions and on almost all mucous membranes directly exposed to the outside; the enumeration of the authors seems unnecessary. The secretion of nutritionally important milk has been examined by me previously (2) and was also observed recently by de Vries and Strikwerda (14). For the sake of completeness it should be mentioned that the ewe discussed at that time still shed listeria with the udder secretion 2½ years after infection in connection with a false pregnancy — she was sterile and did not admit anymore. The listeria were limited to the udder and were found only there even after slaughtering; the blood titer was H20/0160, the germ count in the milk was 2-20,000 per ccm, at times quite low or altogether absent.

The cisternal infection of cattle, also described already, had led to sacrifice after settlement of the pertinent quadrant with listeria for 13 weeks. Later we tried to infect a second lactiferous cow by way of the blood stream, similarly to sheep. The animal received intravenously 50 ccm of broth culture of strain 1604/IVA isolated from a bovine fetus, and reacted with a short climb in temperature to 40.9°C and a maximum titer of H220/0640 on the 9th day after infection. On the 16th day after infection we were able to demonstrate listeria in the urine, but the udder and milk remained unchanged, i.e. this time no settlement by germs in the udder occurred, as had been the case with sheep. Three weeks after infection we again infected one udder quadrant of the animal cisternally and saw 24 hours after infection the first signs of mastitis. The teat was increasingly warm and sensitive, the milk flakey and with an increased cell content. Starting on the second day the infected quadrant was slightly swollen for 1 week, coarser and warmer, but not sensitive, and the yield of milk was decreased by one half. Listeria were found only on the second and third days after infection, in small amounts in the milk. The cell count in the infected quadrant was strongly increased and contained about 70% polymorphonuclear leukocytes, up to the 16th day after infection when the animal was sacrificed. The leukocyte content of the blood, which had not suffered a change by the intravenous infection, rose from 5-8,000 to 8-14,000 following the galactogenous reinfection, without change in the cell picture, and the blood titer rose to H320/01280. On the 6th and 9th days after infection we again found listeria in the urine, while they could not be demonstrated in the other secretions and excretions. After slaughtering, which took place 5 weeks after the first infection and 16 days after reinfection, listeria could be found only in the kidneys, despite enrichment under low temperatures and examinations under slanted light. The udder, including its lymphatic glands, must have rid itself of the infection. This view is supported by the absence of prolonged secretions via the milk. It remains to be determined on a large scale whether cattle basically react differently from sheep, as evidenced by our failure to infect the udder hematogenously.

Occasionally we also found listeria in the feces of our test animals, which they probably reached via the liver. As a rule septicemically ill mice, if they weather the infection, excrete listeria in the feces for about 1 week after infection, otherwise up to the time of death. Aside from the secondary occurrence of listeria in the liver of swine afflicted with plague (1), we also

found listeria in the liver of weaned lambs suffering from cerebral listeriosis. Although Rubarth and Wollarz (11) regularly found listeria in the liver of sucking calves, we were never able to demonstrate these germs in the liver of 150 sucking calves. This also speaks against a wider distribution of listeria-mastitis in our research area. Likewise, we have never been able to determine listeria at examinations of a number of milk specimens from listeris flocks of sheep, although in some cases milk from the mothers of weaned lambs with listeriosis was involved. Although listeria occur sporadically among man and beast, clinical disease forms are met frequently only in sheep. We attribute this to the maintenance of flocks with poor accommodation in frequently inadequate or makeshift stalls and to frequently substandard nutrition.

Since other authors also occasionally found listeria in the organs or the musculature of animals slaughtered because of listeriosis, the animals must be examined bacteriologically or boiled. Isolated germs found at times even in healthy animals should be unimportant, because we ascribe only a moderate pathogenicity to listeria, although the contagiousness is considerable.

Outside of the animal's body we have not been able to demonstrate listeria in the surroundings of sheep afflicted with listeriosis, i.e. in the dust, manure, etc. Such tests benefit greatly by the observation of plates under obliquely falling light, as recommended by Gray. From a number of tests in progress on the durability of listeria in the external world only one indication may be alluded to at this time: That the germs remain viable for more than 4 weeks in dry or moist manure or dried within albumin-rich dirt. They die within a few days in water as well as in pure water and foul water aquaria populated by animals and plants. However, if a small amount of meat is placed in the aquarium the germs can be demonstrated for weeks with varying success. Salmonella proved to be considerably more durable in water under the same conditions.

As is well known, listeria reproduce rapidly in albumin-rich milk. For this reason we have conducted tests, discussed at length by Schulze (12) in a dissertation, in which the resistance of listeria was again carefully examined, utilizing my capillary method as compared to the usual milk heating method, and applied to numerous strains. Listeria suspended in milk withstood 65°C for 30-40 seconds, 75°C for 10 seconds, and 85°C for about 1 second, as our published diagram (3) readily discloses. The divergent results of other authors can be explained as being due to utilization of different methods.