TRANSLATION NO. 268

DATE: July 1968

AD 683014

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Experimental airborne infection with listeria of some species of animals.

by V. I. Popov. - From the laboratory engaged in the study of the diseases of swine, VIEV (All-Union Institute of Experimental Veterinary Medicine) Scientific Supervisor: Professor P. S. Solomkin.

Collected Works. All-Union Experimental Veterinary Institute. pp. 95-105. 1957.

To the present time the reservoir of the listerellosis pathogen in nature has not been conclusively established. Its paths of penetration into an organism and the periods of its retention and excretion by sick and convalescent animals are questions that remain unclear.

The majority of investigators, while not rejecting the airborne path, consider the oral path of infection as the more probable.

It is known that the listerellosis pathogen can remain viable for a long period in forage, manure and dust, retaining its pathogenic capacities.

A case in Norway was described where a peasant, while cleaning sheep pens where sick animals were located, was infected with listerellosis by dust containing the disease pathogen.

Dedie, K. (1955) on the basis of an experiment on white mice, came to the conclusion that the aspiration of an aerosol of listeria leads to the death of the animals from a subacute septicopyemic process.

The fact that an airborne infection is more probable under natural conditions and also the indications of some authors served as a premise for the investigation of this question.

We decided to find out in our experiments the possibility of infecting white mice, guinea pigs and young pigs by the airborne route in comparison with the other methods of infection, and to establish the times of excretion and retention of the infectious material.

For the infection of the mice and guinea pigs we used strain No. 2 of Listerella monocytogenes, and for the infection of the young pigs, a mixture of listeria strains (Nos. 2, 9, 34, 199 and 3501) which were isolated from swine. The cultures grew for 36 hours on MPA at 37°C, were washed off with MPB, and a definite concentration of microbic bodies established in 1 ml of MPB according to the optic standard.

The aerosol infection of the mice and guinea pigs was made in a chamber with a 20 l. capacity, and the young pigs in a chamber with a 321 l. capacity. The chambers were hermetically sealed and had openings in the top for a spraying device and for an air outlet.

The diffusion was made with a medical inhaler, to which was attached a force pump of the factory "Respirator," model AI-1, giving an air stream at approximately three atmospheric pressures.

The size of the aerosol's particles were determined by the I. I. Elkin and S. I. Eidelstein method. It was found that upon diffusion of the MPB (pH - 7.2), particles are formed with sizes from 0.5 to 10 microns. Moreover, the field of vision is quantitatively predominated by the small particles. With a diffusion of a physiological solution of sodium chloride (pH - 7-7.3) the size of the particles ranges from 6 to 30 microns, and the field of vision is predominated by the large particles.

The white mice, guinea pigs and young pigs were placed into the chamber where the dynamic aerosol was created. The diffusion of the suspension of microbes was made fractionally. The density of the cloud was determined by the brightness of a ray of light passing through the chamber.

For the subcutaneous and oral infections the same cultures of listeria were utilized that were used for the airborne infection, and in the same concentration.

In the bacteriological investigation of the internal organs and excreta from the animals which died or were sacrificed, we made sowings on MPB and MPA with 1 % glucose and 2 % glycerol. Together with the usual cultures made with a pipette, portions of the organs were placed into flasks and large test tubes with the medium, and divided cultures on bacterial dishes of a suspension of the organs and excreta were also used. From small pieces of the liver, spleen, lungs, lymph nodes and brains of sacrificed young pigs was prepared a suspension in a physiological solution in a 1:2 dilution, which was injected into the white mice subcutaneously in a 0.5 ml dose.

1. Experiments on the airborne infection of the white mice.

Experiment No. 1. An examination of the possibility of airborne infection of white mice in comparison with subcutaneous and oral infection.

Sixty white mice, weighing 18-20 g, were used in the given experiment. The mice were divided into 3 groups of 20 each.

The mice of the first group were placed for 30 minutes into a chamber where a culture of listeria, after a 36-hour growth on MPA, was diffused; the washing was done with MPB; the density of the suspension of microbes was 500 million bacterial bodies in 1 ml. The diffusion was made fractionally - 1 to 2 minutes after every 4-5 minutes. The mice of the second group was infected subcutaneously with 0.3 ml of this same suspension of listeria. The mice of the third group were infected per os; the culture was given with milk with a calculation of 0.5 ml of the listeria suspension for each mouse.

For substantiation of the received data this experiment was repeated on 51 white mice that weighed 18-20 g. each.

The data from the experiments are shown in tables No. 1 and 2.

It is evident from the cited tables that a 30-minute and a 15-minute stay in the chamber with an aerosol in which 1 or 0.5 ml of a 500-million

suspension of listerella is diffused, constitutes a lethal dose for mice. The latter die in 90-100 % of the cases in periods of approximately 5-7 days. The germs were detected in all the investigated organs and excreta of the animals. In the subcutaneous injection of 0.2 ml of the same suspension of listeria the mice die in 55-70 % of the cases; the germs are always found in the brain and in the otherorgans and excreta, although not in all of the deceased animals. The mice die after longer periods, 13-22 days; the incubation period is also prolonged. (Table 1)

In the oral infection made by feeding the suspension of listeria in the MPB, which contains 500 million microbic bodies per 1 ml and is mixed with milk, in a dose of 0.5 ml, the animals died in 25-55 % of the cases. The germs are always discovered in the brain, and in isolated instances in the other organs; the death of the animals occurs within 9-11 days.

Experiment No. 2. For establishing the DLM, DL-50 and the sublethal dose, experiments were staged with exposures to inhalation of the listerellal suspension of 5, 2, 1, 0.5 minutes and 15 seconds. The mice were not placed in the chamber, but were located for the designated time period at an opening of the chamber from which escaped a sol saturated with listeria.

The results of the experiments are shown in table 3.

These experimental results indicate that in the given case the death rate of the mice from listerellal sepsis after a 50-minute inhalation of a 500-million listerellal suspension is 100 %, a 2-minute inhalation causes 70%, a 1-minute - 60 %, a 0.5-minute - 40-50 % of the cases, whereas in the subcutaneous infection death occurs in 60 % of the cases, and in the 15-second exposure the mice remain alive.

(Table 2)
(Table 3)

As seen, the white mice are extremely sensitive to listerella infection with a penetration of the pathogens through the respiratory tracts. These data become even more convincing if the loss of the sol is taken into consideration. The loss of sol in our experiments exceeds 50%, because a precipitation of the aerosol's particles occurs on the walls of the chamber, on the animals, in the transmittal of the particles through the rubber tube etc.

The mice are less sensitive to the subcutaneous and oral infection and die, for the most part, not from listerellal sepsis, but from the development of an affliction of the central nervous system. Obviously the pathogenesis in the airborne infection is different than with the subcutaneous and oral.

For a check on the harmless [illegible] in the aspiration of a sol not containing listeria, 10 white mice were kept for a 1-hour period in a chamber where MPB was diffused. With this, no divergences from normal were noted in any of the animals.

The majority of investigators consider guinea pigs as a suitable laboratory type for the investigation of listerellosis, whereas, Zeeliger, Pallaske and Ogden point their slight resistance to a subcutaneous or oral infection.

Zeeliger writes that for the reproduction of an infection in guinea pigs, larger doses are required than for other small animals. Farther on he indicates that an intravenous injection causes a lethal disease, whereas, in an oral infection the animals remain healthy. With an instillation of live listeria into the conjunctival sac, the majority of the animals, after recovery from a local conjunctivitis, recovers, although a portion of them may die.

The reproduction of kerato-conjunctivitis in guinea pigs and rabbits is one of the methods of testing the pathogenicity of listeria.

In the literature easily available to us, there is no infection of guinea pigs described.

We arranged 2 experiments (10 guinea pigs in each; their live weight was 220-250 g each.) for a determination of the possibility of infecting guinea pigs with airborne germs. The guinea pigs were infected according to the method described previously. They were placed into a chamber where they were kept for a two-hour period. During this time there was diffused 3 ml of a listerellal suspension of a 500-million concentration per 1 ml.

Ten guinea pigs were infected in the conjunctival sac (2-3 droplets of culture in one eye), and 5 subcutaneously with a dose of 3 ml of this same listerellal suspension.

In the animals infected by the airborne method, on the 2nd-3rd day there was observed a refusal of food, a mucosal discharge from the nose, a quickened breathing. On the 3rd-5th day all of the guinea pigs died.

Upon autopsy there was a strong hyperemia of the lungs, enlargement and blood filling of the liver. In some there were punctate grayish-white focuses in the liver, the spleen was pale.

In smear impressions from the liver, lungs and spleen were detected a large number of gram-positive bacilli.

A growth of Listerella monocytogenes was detected in cultures from all the organs, urine , and the mucus from the nasal cavity.

In the animals which were infected in the conjunctival sac, a strong lacrimation was noted on the 2nd-3rd 24-hour period; a purulent secretion was discharged from the eye, later, a complete closure of the eye fissure, and on the 7th or 8th 24-hour period - a corneal opacity. Some animals refused food during the period of the strong development of the conjunctivitis. On the 15th-20th day the animals regained their health. Within 5-6 24-hour periods after the recovery the guinea pigs were sacrificed and a bacteriological investigation made of their organs.

From the 5 guinea pigs infected subcutaneously, two died from listerellal sepsis, three remained alive.

As a result of the bacteriological investigation it was established that the guinea pigs infected in the conjunctival sac may discharge the listerellosis pathogen with their urine up to 40 24-hour periods and remain carriers of listerellosis.

Guinea pigs are extremely sensitive to the airborne infection and die from listerellosis on the 3rd-5th day in 100 % of the cases. Conversely, they are slightly sensitive to the subcutaneous infection from which they die in 40 % of the cases.

As a control in these tests we used three guinea pigs which were kept for three hours in a chamber where a sterile mpb was diffused.

Not every investigator has been successful in creating an induced infection in swine.

D. Gill (1937) injected intravenously a culture of listeria into four young pigs. An increase in their temperature was noted after hwich the animals recovered.

H. Biester and S. Schwarts infected two pigs intracerebrally with cultures of a sheep strain of listeria. One animal died within 48 hours; however, a culture of the disease's pathogen was not isolated. The other pig died within three days and from the tissue of the organs was isolated a culture of listeria. Two other swine, one of which was infected by a single intraperitoneal injection, and the other by 17 intramuscular injections, were sacrificed when they were in a serious condition. Attempts to isolate cultures of listeria from their organs were unsuccessful, but the histological changes were characteristic for listerellosis.

P. P. Sakharov and E. I. Gudokavaia did not succeed in infecting a pig intramuscularly with a twofold injection of a culture of listeria in 5-6 ml doses.

R. Graham, T. Dunlap and M. Levine (1940) informs of a successful infection through the ocular conjunctiva of a single young pig, with a lethal result. A culture of listeria was isolate from the pig's organs.

R. Hubik and Laznicka (1955) managed to produce intracerebral, peritoneal and intravenous infection.

In our experiments 10 young pigs, weighing 5-6 kg each and 1 to 1.5 months of age, were subjected to infection. For the infection, virulent strains of listeria isolated from swine were used.

In the first experiment pigs No. 13 and 14 were placed into a 321 l. capacity chamber for 5 hours two days runnung. In this chamber was diffused a mixture of the strains of a listerellal culture of a 24-hour incubation on MPA. The washing was made with MPB; the density of the suspension was 5 milliard bacterial bodies per ml. It was diffused fractionally, 30 minutes each time with 15-20 minute intervals; in all 30 ml of the culture was diffused in the 5 hours. In the chamber observation was maintained over the animals, after each hour the number of respiratory movements per minute was calculated and the temperature in the chamber noted. Before placing the animal into the chamber and after its withdrawal the body temperature was checked, the number of respiratory movements and the pulse calculated. During the 10th day after the first infection, blood, urine, feces and nasal mucus were taken for bacteriological reasons and investigated according to the usual method. The data are reflected in tables No. 4 & 5.

(Table 4)

On the following day after the infection the animals refused food, lay down, burrowed into the bedding litter, did not respond to call; the respiration was shallow, quickened, of a bone-abdominal type with moans. Upon auscultation of the chest cavity, moist rales and a cheeping sound were audible. On the 2nd and 3rd day this condition continued, and from the nasal cavity was excreted a viscous yellowish mucus, the conjunctiva was hyperemic. On the 4th day the animals sluggishly accepted food, but their condition was depressed; the rales ceased; mucus was discharged from the nasal cavity in small quantities. On the 5th and 6th day the animals began to accept food, their recovery had begun.

In the second experiment, on two young pigs of the same age and live weight as in the first, the infection was made by the method indicated previously, but the concentration of bacterial bodies in 1 ml was increased to 20 milliard.

As a result of this experiment one pig died from listerellal sepsis on the 3rd day, the other recovered after a serious illness.

In the following experiment, 4 one-month-old pigs were subjected to infection by the previously described method. The animals recovered from the disease with difficulty and remained alive. The bacteriological investigation of the blood, urine, feces and nasal mucus in the pigs of these experiments are analogous to the data received in the first experiment.

(Table 5)

In order to establish the possibility of an oral infection, pigs No. 15 and 3, age 1.5 months, received over a 5 day period 0.5 l. of a 24-hour-old growth of a listerellal culture in broth.

During the two-month observation over them, an emaciation was noted; the bristle was yellow, dull, disheveled.

The blood serum was investigated for RA (agglutination reaction ? -Tr note) with the VIEV antigen in all animals three times prior to infection and every 5-6 days after infection. By this we established that the RA appeared within 5-10 days after infection and was positive in a dilutic of 1:100, 1:200 during the entire period of observation.

In order to determine the continuance of the listeria in the organism, 8 experimental pigs were killed at different time periods and their organs subjected to bacteriological and biological investigation.

The results of the investigation are shown in table 6.

From the cited table it is seen that a pig remains a carrier of listeria for a long period (75 days), the pathogen is localized primarily in the brain and the lungs, which is necessary to take into consideration in a bacteriological diagnosis of the subject disease

(Table 6)

Conclusions

1. White mice and guinea pigs are extremely sensitive to listerellosis in the airborne method of infection and less sensitive to the subcutaneous and oral methods. Where the causative agent is introduced per os or subcutaneously, the majority of the mice die with the clinical manifestations of an affliction of the central nervous system.

2. Excretion of listeria by the sick and convalescent guinea pigs occurs through the nasal discharge, urine and feces for a period of 40 24-hour periods, and by pigs up to the 8th 24-hour period after infection.

3. Pigs become ill with listerellosis upon their infection by an aerosol and remain carriers of listeria as long as 75 24-hour periods after recovery.

4. The frequent detection of listeria in the brain tissue of the experimental animals, without a visible clinic of the disease, indicated a prolonged carrying, and the presence of a hidden form of the listerellal infection, under natural conditions, that should be taken into consideration in a bacteriological diagnosis and in an analysis of an epizootic and epidemiological situation.

Literature

Ellin, I. I., Eidelstein, S. I. - Aerosols of antibiotics, their production and clinical use. Medgiz, 1955.

Sakharov, P. P. and Cudkova, E. I. - Listerella infection (neurellosis). Medgiz, 1950.

Svintsov, P. M. - Listeria and Listerellosis of Animals. Dissertation, dok. 1956.

Khalimbekov, M. M. - Listerellosis of Sheep and Goats in Azerbaijan. Dissertation, dok. 1956.

Diester, H. a. Schwarte L. - Listerella infection in swine. Jour. Amer. Veter. Med. Assoc. 1940. 96, p. 339-342.

Dedie, K. Beitrag zur Epizootogie der Listeriose. Archiv fur Experimentalle Veterinarmedizin, 1955, B. IX. H. 3.

Graham, R., Dunlap, T., and Levine, D. - Experimental listerellosis in domestic animals. The Cornell veterinarian. 1940, v. 30. No. 3.

Hubik and Laznicka - Listeriove infekce prasat. Veterinarstivi, 1955, 10.

Seeliger, H. Listeriose. Leipzig, 1955.

Table 1

Results of the experiment of infecting mice with listeria.

Date of Infection	Method of infection	No of white mice in test.	Dose used in the airborne infection for all animals		Dosage in ml. for ea. animal.	Period of Sickness	No of mice which died	Left alive	Death %age	Investigated material from the animals which died: in the number of cases in which listeria were isolated.								
			Exposure to inhalation in min	Amount of culture diffused in ml.						heart	lungs	liver	spleen	kidneys	urine	feces	nas. mucus	brain
10 Apr	airborne	20	30	1	-	15-24 Apr.	18	2	90	15	18	18	18	15	12	9	18	18
	subcutan	20	-	-	0.3	15 Apr-7 May	11	9	55	5	2	7	8	4	3	3	-	11
	alimentary.	20	-	-	0.5	14-20 Apr	5	15	25	4	2	5	5	2	2	2	-	5

Note: The animals were under observation for a period of 30 24-hour periods. Those remaining alive were killed and their organs subjected to a bacteriological investigation.

Table 2

Results of the experiment of infecting mice with listeria.

Date of Infection	Method of infection	No of white mice in test.	Dose used in the airborne infection for all animals. Exposure to inhalation in min	Amount of culture diffused in ml.	Dosage in ml. for an. animal.	Period of sickness	No of mice which died.	Left alive	Death rate	Investigated material from the animals which died: in the number of cases in which listeria were isolated. heart	lungs	liver	spleen	kidneys	urine	feces	Mes. mucus	brain
28 Apr	airborne	20	30	1	-	30 Apr-7 May.	20	-	100	20	20	20	19	19	8	10	20	20
	"	11	15	0,5	-	2 Apr-7 May.	11	-	100	10	11	11	11	11	4	5	11	11
	subcutan.	10			0.3	1 May-14 May.	7	3	70	4	4	4	4	4	1	3	1	7
	alimentary.	10	-	-	0.5	3 May-14 May.	5	5	50	1	1	1	1	1	1	1	-	5
	Total	51					43	8		35	36	36	35	35	14	19	32	43

Table 3

Results of the infection of mice with listeria.

Date of Infection	Method of infection	No of white mice in test.	Dose used in the airborne infection for all animals. Exposure to inhalation in min	Amount of culture diffused in ml.	Dosage in ml. for ea. animal.	Period of sickness	No of mice which died.	Left alive	Death rate	Investigated material from the animals which died: in the number of cases in which listeria were isolated. heart	lungs	liver	spleen	kidneys	urine	feces	nas. mucus	brain
24 May	airborne	11	5	0.3	-	30 May-31 May.	11	-	100	11	11	11	11	11	5	7	11	11
	subcutan.	5	-	-	0.3	20 May-30 May.	3	2	60	3	3	3	3	3	3	3	-	3
3 Oct	airborne	10	2	0.06	-	9 Oct-15 Oct.	7	3	70	6	7	7	7	6	5	2	6	7
	"	10	1	-	-	9 Oct-1 Nov	6	4	60	6	6	6	5	6	4	-	6	6
	"	5	0.5	-	-	17-22 Oct.	2	3	40	1	1	1	1	1	1	1	2	2
6 Dec	"	10	0.5	-	-	19-25 Dec.	5	5	50	4	5	5	5	3	2	1	4	5
	"	10	15 sec.	-	-		-	10	0									
	Total	61					34	27		31	33	33	32	30	20	14	29	34

Table 4

Temperature, respiration and pulse in the young pigs infected with listeria.

	Pig No. 13			Pig No. 14		
		per min.			per min.	
	T°	Resp.	Pulse	T°	Resp.	Pulse
Prior to placing the animals into the chamber.....	40.2	38	112	40.1	32	120
Immediately upon withdrawal from the chamber.....	40.3	42	140	40.4	48	140
On the following day after the first infection.....	41.6	64	142	41.2	58	154
On the 2nd day immediately after withdrawal from the chamber.....	41.6	76	160	41.2	82	140
On the 3rd day.....	41.6	56	128	41.1	60	148
On the 4th day.....	39.8	50	124	40.5	54	160
On the 5th day.....	39.2	20	118	39.3	22	120
On the 6th day.....	39.6	22	120	39.6	20	128

Table 5

The results of the bacteriological investigation of the blood, urine, feces and nasal mucus from the young pigs infected with listeria.

When the investigation was made.	Pig No. 13				Pig No. 14			
	blood	urine	feces	nasal mucus	blood	urine	feces	nasal mucus
Prior to the infection (threefold).....	-	-	-	-	-	-	-	-
Within a day after the infection.....	+	-	-	+	+	-	-	+
On the second day.....	-	-	-	+	-	+	-	+
On the third day.....	-	-	-	+	-	-	-	+
On the fourth day.....	-	-	-	+	-	-	-	+
On the fifth day.....	-	-	-	-	-	-	-	+
On the sixth day.....	-	-	-	-	-	-	-	-
On the seventh and eigth day..	-	-	-	+	-	-	-	-
On the ninth and tenth day....	-	-	-	-	-	-	-	-

Table 6

Results of the bacteriological and biological investigations on the organs of the young pigs which were sacrificed

Nos of the animals	Method of Infection	The day killed	FROM WHICH THE LISTERIA WERE ISOLATED											Lymphatic nodes								
			heart	liver	lungs	spleen	kidneys	urine	encephalon	spinal cord	medulla	bile	uterus, testicles	submaxillary	retropharyngeal	mediastinal	portal	mesenteric	inguinal	tonsils	nasal cavity
16	airborne	3 (died	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
18	"	30	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
20	"	30	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	"	50	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
3	alimentary	60	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
15	"	75	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
17	airborne	75	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
14	"	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-