TRANSPLACENTAL (PASSIVE) TRANSMISSION OF PLAGUE ANTIBODIES IN THE GREAT GERBIL.

SEROLOGICAL INVESTIGATIONS IN PLAGUE

M. I. Levi and Yu. G. Suchkov
Rostov-on-Don Plague Research Institute
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
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The facts regarding the transmission of immunity from immune female mammals and birds to their offspring are well known in respect of various bacterial and virus infections. Towards the end of the last century Ehrlich demonstrated the passage of antitoxins from immune females to their young. He also found that in mice transmission of antibodies may occur through the mother's milk. A full survey of the research undertaken outside the Soviet Union during the present century on this subject has been made by I. I. Mechnikov [5]. In 1959, N. A. Demina published a survey entitled: "The transmission of antibodies through eggs laid by immune birds." Every year new work concerned with the passive transmission of antibodies by mammals and birds to their offspring is published in the litereature. Recently, for instance, Coffin, Hook, and Muschel [6] have shown that the blood of the human fetus possesses bactericidal activity agains staphylococci, streptococci, salmonellae, and Escherichia coli. D. K. L'vov and R. L. Naumov [4] found antibodies against the virus of tick-borne encephalitis in the offspring of spotted blackbirds. The transmission of antibodies from immune females to their offspring has been reported in measles and influenza, and in all neurovirus infections except lymphocytic choriomeningitis. In the last case, as Weigand and Hotchin showed [7], complement-fixing antibodies are not transmitted from immune albino mice to their offspring. However, we could find no reference in the literature to the transmission of plague antibodies to offspring.

We have studied the passive transmission of plague antibodies to the offspring of the great gerbil. Experiments were carried out on this species (which is a natural carrier of plague) in the Central Asian desert focus of the disease (Northern Aral Territory).

EXPERIMENTAL METHOD

Pregnant great gerbils were trapped in May 1962 in a territory where plague was epizootic. The animals were anesthetized with ether and blood taken from the heart by means of a syringe. Immediately after the blood had been taken some animals were sacrificed and the fetuses, placenta, and amniotic fluid of each fetus extracted separately. The fetus was thrice washed with physiological saline and then carefully ground in mortar with sand, and the mince diluted 1:10 with physiological saline, to give a suspension regarded as equivalent to a dilution of fetal serum of 1:20. The placenta was dealt with in the same way. The third washing fluid was tested for the presence of antibodies by the passive hemagglutination reaction (20 observations), but no antibodies were detected in any of the tests.

After removal of the blood sample, some of the females were left in 10-liter glass jars until parturition. The gerbils were fed on their customary diet Haloxylon ammodendron, Salsola soda, Artemisia, Poa serotina—shrubs growing in the desert region). During the first 24 h after giving birth to their offspring, the females ate them (12 observations). Only 2 of the 9 females kept in dark metal cylinders ate their offspring.

Blood from the newborn gerbils was investigated on the day of birth and on the 5th, 14th-15th, and 20th days thereafter. Blood could not be withdrawn from the gerbils on the day of birth, so that the heart and liver were incised and washings of these organs were obtained with 2 ml physiological saline. These washings were poured into a test tube which was allowed to stand at room temperature for 2-4 h; the upper layer of fluid was removed and taken as fetal serum in a dilution of 1:40. Blood was taken from the heart of the gerbils aged 5 days or more after opening the thorax; the serum of the young gerbils was always opalescent.

TABLE 1. Distribution of Antibodies in Pregnant Females, Embryos, Placenta and Amniotic Fluid

Animal No.	Fluid tested							
	Blood of pregnant female	Suspensions of embryonic tissues			Suspensions of placental tissues		Amniotic fluid	
	Titer	No. of embryos tested in PHR	Mean length of fetus (cm)	Titer	No. of placentas	Titer	No. of specimens of amniotic fluid	Titer
1	5 120	7		640—1 280	7	320—640	5	16—32
2	1 280	4		320—640	4	160—320	4	32—128
3	320	6		320—640	6	20—80	6	<16
4	1 280	6		320—1 280			3	64—128
5	20 480	5		5 120—20 480	5	5 120—10 240	2	2 560—5 120
6	320	3		80—160	3	80—160	3	32—64
7	2 560	5		320—640	5	640	5	128—256
8	320	4	2,5	160—320	4	160—320	4	128
9	5 120	10	1	640—1 280				
10	163 840	9	1	2 560—5 120				
11	5 120	8	2,5	1 280—2 560				
12	10 240	8	2	1 280—2 560				
13	1 280	4		320—640				
14	2 560	9	1	320—640				
15	640	6	2	160—320	6	160—320	6	64—128
16	2 560	6	1,5	1 280—2 560				
17	2 560	4	2	1 280	4	320—640	4	128—256
18—31	0	60	1—2	0	27	0	23	0
Total		164			71		65	

Note. The term antibody titer implies the reciprocal of the limiting serum dilution active in the PHR.

The suspensions of fetus and placenta, the washings of the heart and liver of the newborn gerbil, and the amniotic fluid and the serum of the young and fully grown gerbils were heated for 30 min at 56° and then tested by the passive hemagglutination reaction (PHR) with formalized erythrocytes sensitized with fraction 1A of Pasteurella pestis. The technique of the reaction was fully described in the book: "A Short Textbook of Epizootological Investigation" (edited by A. K. Shishkin, Rostov-on-Don, 1962). The PHR was performed simultaneously on all the material from one gerbil.

EXPERIMENTAL RESULTS

Altogether 31 pregnant females were obtained, of which 17 were found to have antibodies to P. pestis in the PHR. The amniotic fluid and placenta could be tested from only a proportion of these animals, whereas embryos from all the gerbils were tested.

Hence, 104 embryos, 44 placentas, and 42 specimens of amniotic fluid were obtained from the 17 pregnant females with antibodies in their blood, and apart from 6 specimens of amniotic fluid from one gerbil they all contained plague antibodies. In most cases a definite correlation was noted between the antibody level in the female and in the embryos, placentas, and amniotic fluid. In contrast to the dilutions of maternal serum, the dilutions of fetal suspensisons and placental suspensions only approximately reflected the titer of antibodies in the fetal blood serum, a fact which could be explained by the technique used to prepare the suspensions. We were struck by the fact that the antibody titers in the different embryos, placentas, or specimens of amniotic fluid obtained from the same female varied within very narrow limits.

The results of the PHR performed on 60 embryos, 27 placentas, and 23 amniotic fluid specimens taken from mothers having no antibodies in their blood was negative.

Because the great gerbils giving birth to their young in captivity devoured them, we were unable to study the duration of the period in which antibodies were present in the newborn gerbils in a large series of cases, although this could be done occasionally. Altogether 42 animals were investigated.

TABLE 2. Determination of Antibodies in the Sera of Pregnant Females and Their Young

Animal No.	Time of determination before parturition (day)	Antibody titer in pregnant female's serum	Antibody titer in serum of offspring: on day of birth	after 5 days	after 14-15 days	after 20 days
1	12	20 480	1 280, 640*			
2	7	2 560	1 280, 1 280		1 280	640, 640
3	21	320	320, 160			
4	5	10 240	2 560, 1 280	1 280		1 280
5	5	2 560	640, 640	640		160
6	8	640	640, 320	160	160	160, 160
7	3	5 120	1 280, 640			
8	3	160	320, 160			
9	8	5 120	640, 640	320, 640	80, 80	
Total		9	18	5	4	6

*Two figures in a column means that 2 gerbils were tested at this period.

It will be obvious that antibodies were found in all cases when the offspring were tested, and that as a rule a correlation was observed between the antibody titers of the pregnant females and of their offspring. It should not be forgotten that the technique of investigation of the newborn gerbil did not allow the antibody titer in the animal's serum to be determined accurately. For a period of 20 days, high levels of the antibody titer continued to be observed in the offspring.

The results show convincingly that in natural conditions antibodies are transmitted from immune female great gerbils to their offspring. It is not yet certain for how long the passively transmitted antibodies remain in the young gerbils and whether or not they play a protective role. It is stated in the literature that in many infectious diseases the average length of stay of antibodies in the offspring is 2-4 weeks. The length of stay of antibodies in the offspring certainly depends on their initial litter. For this reason we decided not only to determine the length of time during which antibodies could be detected, but also to estimate the half-life period of the passively transmitted antibodies, which is independent of the initial titer. For instance, in the children of mothers immune to the virus of poliomyelitis, the half-life period of the virus-neutralizing antibodies is 37 days [1]. In our small series of cases we could not determine the rate of destruction of passively transmitted antibodies accurately, but the impression gained was that the antibody titer showed a definite decrease at the 20th day.

Various workers have demonstrated the protective role of antibodies in plague [3]. Although there is no evidence yet available showing that passively transmitted antibodies have a protective role, we may submit the following hypothesis. In the natural breeding ground of plague in the Central Asian desert, great gerbils are the principal hosts of the plague microorganism, a factor largely responsible for the relatively high resistance of the adult animals to this infection. In all probability resistance of the great gerbils to plague, like that of the gerbils of the left bank of the Volga, is transmitted hereditarily from generation to generation. However, the great gerbils are not born resistant; this property is formed in the young animals when they stop feeding on their mothers' milk, or even not until the period of sexual maturation. Until the formation of this inherited resistance, in places where plague epizootics reach a high intensity and where the chance of infection of the young animals from the bite of a plague flea is high (it must be remembered that fleas feed more avidly on newborn and young gerbils), they are under the protection of maternal antibodies. In epizootic areas the percentage of adult gerbils whose serum contains plague antibodies varies from 20 to 100. The passive transmission of antibodies from immune females to offspring thus ensures the survival of the young great gerbil in areas with a high incidence of plague epizootics.

SUMMARY

Passive transmission of plague antibodies from immune female great gerbils to their progeny was established. A high antibody titre was revealed in all the embryos, the placenta and amniotic fluid. The antibodies were retained in the serum of the young up to the 20th day after birth (observation period).

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