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## Study of different methods of laboratory investigation of hydrophobia in comparative aspect

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### Abstract

Hydrophobia – dangerous infection disease of animals, birds and persons, which appears hard affection of nervous system, as flying outcome. In spite of success of modern sciences a large territory of the world is not free from the virus of hydrophobia. World areal of hydrophobia of production live-stock has no tendency to reduce it as for natural hotbed of disease.

**Keywords:** Hydrophobia, pathogenesis, infected, inoculation, intracerebral, intraplantar

### Introduction

**Actuality of the theme:** The investigation of hydrophobia of animals in different areas of the republic which investigated at Uzbekistan scientific investigation of Veterinary Institute. The results of this during the last year's show that spreading of this disease among agricultural animals and also plays a great role of wild raft toxic animals as foxes, jackals and wolfs, and also in other wild types of animals. For the sake of that hydrophobia of domestic animals are often infected in country-side, especially during pasturable period.

The speed of spreading virus of hydrophobia into central nervous system from the bits determines pathogenesis of disease and affectivity of treating and prophylaxis. Last times in literatures we came across different opinions about the question.

We studied the time of penetration of virus of hydrophobia into central nervous system on the base of location of tamp on the body of different types of animals.

**Materials and methods:** We had six series of experiments on 36 foxes, 36 dogs, 36 jackals, 60 white mice, 36 rabbits, 48 domestic mice and 48 grey rats.

For infecting all experienced animals was used epizootic stamp (S-33) on rabbits of street virus of hydrophobia, singled out from sick with hydrophobia jackals with titre 6, 0-6, 6 LD<sub>50/0,03</sub> ml.

According to the diagram of experiment we have done preparations from tissue of fox, we came from central nervous system of dead experienced animals. Besides those, we bio tested 6 white mice, infecting their brains with 0,03 ml of 10% virus containing suspension, prepared from tissues of mice of brain. On the results of that we noticed the virus with which infected the hip of the dogs which can be discovered in 24 h, on the fox – in 24-48 h, on jackals – 48 h, and then the virus may be discovered in brains in seven, eight, nine days.

On the second type of experience on 16 foxes, dogs and jackals which was led 10% virus containing suspension of rabbit brain with the size of 3 ml physiological solution which was led into two points of chewing tissue in one time. Experience on the base of described method as it is given on page 2 on the results of investigation was fixed that the virus on the dogs was determined in 12 days after infection, in brain – after 6 days. But we couldn't find out infected tissue after 24 h. and on the foxes virus was discovered till 24 h., in brain – till 7 days. After infecting jackals with virus of hydrophobia was discovered till 24 h., in brain – till 8 days.

During the third experience we used 30 white mice and 18 rabbits. We infected those animals with 10% virus containing suspension of rabbit brains on physiological solution, under the skin of tip of the nose in the size: 0,03 ml on white mice, 0,2 ml on rabbits and investigated the presence of virus hydrophobia on the base of described methods. On the results of investigation we singled out that infected virus was kept till 12 h, in brain – till two days in

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White mice. But in rabbits virus of hydrophobia was discovered in 12 h, in brain – till 3 days. In 24 hours and more time it was not available to reveal virus in infected place of rabbits.

As it is seen on the results of the experiment virus on domestic mice was kept in active state till 12 hours after infected, in brain can be revealed for 3 days. In 24 hours and more period of time virus in infected muscle was not revealed. And on grey rats can be discovered after 12 hours, in brain can be revealed on the 5<sup>th</sup> day.

The sixth series of experiences of 30 domestic mice and 30 grey rats which were infected with 10% virus containing suspension of rabbit brain with physiological solution in muscles of thigh was infected with 0,1 ml and 0,5 ml and 3 of animals of every type were dead after infecting in 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 hours which were investigated on the base of the method in the presence of hydrophobia virus. On the results of this we singled out that after infecting with virus in domestic mice we discovered in 24 hours, in brain – on the 5<sup>th</sup> day. In the latest period of time it is not available to reveal virus in infected muscles.

On the sample of hydrophobia virus of grey rats can be discovered in 12 and 24 hours after infecting, in brain – in 5-6 days. Investigation of virus in infected muscles is not discovered in other periods of time.

So, the experiment on white and domestic mice, grey rat, rabbits, foxes, jackals and dogs were shown in different

periods of time the revealed hydrophobia virus in brain from infected animals, on the dependence from the place of its inoculation. At any case in 12-48 hours virus was not available to single out of the infected place by different methods. In brain hydrophobia virus was singled out in different periods of time i.e. from 3 till 10 days. The results of investigation which we got have definite clearness pathogenesis of illness in different animals on the dependence of used methods.

The hydrophobia virus of three types were investigated on 15 karakul sheep of both sex, the age of 1,5-2 years, dividing into 3 groups, each group consisted of 5 sheep. Sheep were bound in normal place for hydrophobia; blood serum wasn't antibodies to the virus of hydrophobia.

After 30 days of quarantine sheep was infected 10% in brain containing virus of hydrophobia in different isolation as S-185, L-125 and S-191, on the level of 2-3 passage.

Virus containing suspension were passed into chewing muscles of three group sheep in the size of 2,0 ml, by 1,0 ml on each side. Infecting titre of experimented insulations became equal from 6,5 till 7,5 lg LD<sub>50/0,03</sub> ml in infected white mice in the size of 6-7 gr. We examined infected animals during 3 months. Results of investigation is given in the diagram 1.

Diagram 1.

**Table 1:** Pathogenesis of street virus of hydrophobia of karakul sheep

Insulator virus hydrophobia	Titre virus	Infected animals	Palo of animals	Number of animals	Continuation (days)		Form of disease	Death after infection
					Period of incubation	Clinic exertion		
S-185	7,2	5	5	1	9	2	Paralysis	11
				2	12	2	Paralysis	14
				3	14	3	Paralysis	17
				4	12	2	Paralysis	14
				5	12	2	Paralysis	14
L-125	7,5	5	5	6	8	3	Paralysis	11
				7	12	2	Paralysis	14
				8	13	2	Paralysis	15
				9	10	3	Paralysis	13
				10	9	2	Paralysis	11
S-191	6,5	5	5	11	0	0	-	0
				12	27	3	Paralysis	30
				13	39	2	Paralysis	31
				14	0	0	-	0
				15	10	4	Paralysis	44

It is seen from the diagram, that the incubated period of sheep, infected S-185 were from till 14 days (average 11,8), in the second group of sheep, infected insulation L-125 during 8-13 days (average 10,4 days) and in the third group of sheep, infected insulation S-191 27-40 days (average 35,3 days). On the results of this ten sheep infected insulation S-185 and L-125 were dead, five sheep of infected insulation S-191 three sheep were dead, which were ill after long incubated period, continuing 10-39 days, two sheep of this group were stable to infection.

Experimental infected sheep with hydrophobia virus and their illness was in paralytic form, it was clinically appeared during 2-4 days. The main symptoms were expressed in apathy, tremor, salivation and paralysis.

The results of infected sheep with hydrophobia virus allow us to notice that two insulation (S-185, L-125), which were singled out in stationery bad zone, were seemed high pathogenesis for sheep, and insulation S-191, singling out in stationary conditionally good zone – less pathogen. These data allow us to conclude, that in the territory of Uzbekistan circulate stamps of street virus of hydrophobia, having different degrees of pathogenesis in infecting different types of animals.

While investigating the accumulation of virus in brain of infected sheep with insulation S-185, pointed out titre 5,6-6,1 lg LD<sub>50/0,03</sub> ml and with insulation L-125, the titre was 5,3-6,0 lg LD<sub>50/0,03</sub> ml and with stamps of hydrophobia virus S-191 the titre was fluctuated 2,5-3,9 LD<sub>50/0,03</sub> ml.

On the 90<sup>th</sup> day after infecting 2 not diseased sheep killed and were examined with virological investigation. Hydrophobia virus in brain of those sheep by means of bio testing method and MFA was not discovered.

For investigation of pathogenesis of stamps of street virus of hydrophobia of donkeys were experimented on 24 donkeys with the age of 1, 5-2 years old. The donkeys were bound from stationery good into having hydrophobia zone, before infecting they were in quarantine for 45 days. In blood serum slavish imitation of antibody was absent. For infecting donkeys we used 10% tissue brain of white mice, infected by insulation of hydrophobia virus – S-185, L-122, S-191, L-125.

Experimented donkeys virus containing suspension, in one group we operated into chewing muscle and in others (12

donkeys) - under the skin of neck is each side in the size of 2, 5 ml which consisted of 5, 0 ml. The infected donkeys were controlled during three months. The results of infecting are given in diagram 2.

It is seen on the diagram 2 that the donkeys, infected inner muscle insulation of hydrophobia virus S-185 and L-125 were ill and dead. Incubated period of illness was 12-19 days (average – 15,5 days). The donkeys after infecting under the skin with those stamps they were ill and dead. In this case incubated period in both cases consisted of 18-25 days (average – 21,2). Clinical features of infected donkeys were observed during 2-3 days.

Diagram 2.

**Table 2:** Pathogenesis of stamps of street virus of hydrophobia of donkeys in different ways of infecting

Insulator virus hydrophobia	Titre of virus 1 g LD <sub>50/0,03</sub> ml	Infection of inner muscle		Infection of under skin	
		Infected and Palo of animals	Incubated period (days)	Infected and Palo of animals	Incubated period (days)
S-185	7,0	3/3	12-17	3/3	18-23
L-125	7,3	3/3	14-19	3/3	19-35
S-191	6,5	3/2	29-39	3/1	41
L-122	5,5	3/1	45	3/0	0

Three donkeys were infected inner muscle insulation S-191, from which two were dead. Incubated period lasted 29-39 days (average 34 days). In infecting under the skin with the same stamps one of three was ill, incubated period lasted 41 days. Illness lasted for 2-4 days. From three donkeys which were infected inner muscle stamps L-122, only one was dead. Incubated period was 45 days. Illness lasted for 5 days. To stamps L-122, donkeys which were infected under the skin seemed stable and during 90 days control animals were not ill.

Accumulation of virus in brain of donkeys, infected inner muscle insulation S-185 and L-125 was high and its titre was 5,0-5,5 lg LD<sub>50/0,03</sub> ml, and in brain of donkeys infected with these insulation under the skin, titre of virus was 3,0-4,1 lg LD<sub>50/0,03</sub> ml.

Titre of virus in brain of donkeys, infected with inner muscle insulation S-191, was 2,1-3,0 lg LD<sub>50/0,03</sub> ml, in infecting with these insulation titre of virus was 0,03 ml, 1,5 lg LD<sub>50/0,03</sub> ml and after infecting inner muscle with insulation L-122 titre of virus was equal to 2,0 lg LD<sub>50/0,03</sub> ml.

On the 90<sup>th</sup> day after infecting 8 donkeys which were not ill they were killed and had to test to virological investigation of brain with the methods of using bio testing on young muscles and MFA. We were not able to distinguish virus from brain of those animals.

So, the investigated 12 insulation of hydrophobia virus, we singled out in the territory of Uzbekistan from fox, jackal, wolf, red gopher, domestic and field mice and bat turned out to be pathogenic for white mice and white rats at infecting in brain, intraplacentally and hypodermically. The investigated 23 insulation of hydrophobia virus there were also singled out from fox, jackal, wolf, domestic and field mice, grey rat, dog and cattle and they turned out to be pathogenic for rabbits at using it hypodermically, into muscle and abdominal cavity. There were studied 4 types of street viruses at experimental infecting dog, sheep and donkey inner muscularly. 2 insulation of hydrophobia virus, singled out from dog and fox turned out to be highly virulent. One of the insulations singled out from foxes, was virulent and weakly virulent, and hypodermically infecting donkeys it turned out to be virulent. Red gophers and eagles didn't react on infecting, but they were bearers of the virus for a long time.

Mentioned above wild and domestic animals, according to the results of investigation, are potential sources of hydrophobia virus in the territory of Uzbekistan.

In conclusion above pointed investigation and experiments are given diagram 3.

Diagram 3.

**Table 3:** Comparative investigation of sensibleness and specification of different methods of laboratory diagnostics of hydrophobia

Types of investigated animals	All investigated materials	Positive results									
		MFA		Bio test		MFA + bio test		RDP		Light microscope	
		Revealed	%	Revealed	%	Revealed	%	Revealed	%	Revealed	%
1.Devision of hydrophobia: K RS	31	23	74,0	23	74,0	23	74,0	14	47,5	11	36,0
Small cattle	20	8	40,0	8	40,0	8	40,0	5	25,0	4	20,0
Dogs	67	51	76,1	51	76,1	51	76,1	33	49,2	27	40,0
Cats	13	5	38,4	5	38,4	5	38,4	2	15,3	2	15,3
Horses	9	6	68,6	6	66,6	6	66,6	4	44,4	3	33,3
Donkeys	16	4	25,0	4	25,0	4	25,0	3	18,7	1	6,2
Wolves	21	7	33,3	7	33,3	7	33,3	4	19,0	4	19,0

Foxes	20	9	46,0	10	50,0	10	50,0	5	24,8	3	15,0
Jackals	13	5	38,4	5	38,4	5	38,4	2	15,3	2	15,3
Badgers	5	2	40,0	2	40,0	2	40,0	1	20,0	1	20,0
Animal total:	215	120	56,0	121	57,0	121	57,0	73	34,7	58	27,4
2.Experimental infected: dogs	19	19	100,0	19	100,0	19	100,0	12	68,4	11	57,9
Foxes	18	17	94,4	18	100,0	18	100,0	10	55,5	6	33,3
Jackals	5	5	100,0	5	100,0	5	100,0	2	40,0	2	40,0
Grey rats	12	12	100,0	11	91,6	12	100,0	7	58,3	5	41,6
Animal total:	54	53	98,1	53	98,1	54	100,0	31	57,4	23	44,4

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