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From the Russian for
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[Tularemia] 89-94, 1947.

Zhidkaya zheltochnaya sreda dlya vyrashchivaniya kul'tur tulyaremii
Soobshcheniye IV. Primeneniye zhidkoy zheltochnoy sredy dlya uskoreniya bakteriologicheskoy diyagnostiki tylyaremii

A liquid egg yolk medium for growing Pasteurella tularensis.

Report IV. Liquid egg yolk medium used as a means of accelerating the bacteriological diagnosis of tularemia.

by

M. S. Drozhevkina

It is generally known that when rodents are checked for tularemia, the isolation of cultures presents considerable difficulties, The biological test method has been widely adopted in practice for the investigation of rodents only because cultures of the agent cannot be successfully isolated by inoculating culture media with it. Furthermore, the methods employed thus far for testing rodents by isolating Bact. tularense are extremely slow. The inadequacy of the present bacteriological methods makes it imperative to find simpler and quicker methods.

Experiments having demonstrated that the tularemia agent can be easily grown in liquid egg yolk medium from small inoculation doses, and that it proliferates rapidly in this medium, the author proposed to find out whether the medium in question could not serve to accelerate the bacteriological diagnosis of tularemia in rodents.

Isolating Bact. tularense cultures in the liquid egg yolk medium from rodents dead of tularemia. It was demonstrated in preliminaty studies (Report I) that Pasteurella tularensis could be isolated more rapidly and with better results by inoculating the liquid egg yolk medium directly with organ fragments from dead animal sources, than by attempting to grow it on McCoy's marmots. The experimental results duplicated those obtained with the laboratory animals.

In the tests on wild rodents, bacterial growth generally started within twenty-four hours, while on McCoy's medium it did not start until after a 2-3 day waiting period. Besides, material from some of the organs failed to produce cultures on McCoy's medium. Table I contains some characteristic data referring to marmot No. 350, and house mouse No. 310, which had died having been experimentally infected with tularemia.

Table I.

Pasteurella tularensis cultures obtained from organ fragments from animals dead of tularemia seeded directly into the liquid medium.

Animal, species and no.	Medium	Growing time in days	Material inoculated				
			Lymph	Spleen	Liver	Lungs	Blood
Marmot 350	Liquid egg yolk	1	+	+	+	-	-
		4	+	+	+	+	+
"	McCoy's medium	1	-	-	-	-	-
		4	-	+	+	-	-
House mouse 310	Liquid egg yolk	1	+	+	+	+	+
		4	+	+	+	+	+
"	McCoy's medium	1	-	-	-	-	-
		4	+	+	+	-	+

Symbols: + = bacterial growth; - = no bacterial growth.

The same experimental results were obtained with live animals in various stages of tularemia. For example, several groups of marmots were infected with different doses of Pasteurella tularensis strain No. 9 and it was found (as in some experiments on white mice) that the superiority of the liquid egg yolk medium over the McCoy medium is even more evident with smaller inoculation doses. Table II presents data on experiments with marmots infected with single doses of 5 million bacterial bodies.

Several groups of house mice infected with tularemia were investigated by the same method. The results were the same as those obtained with other types of rodents (Table III).

On summing up the results of the latest series of experiments, the author concludes that for purposes of bacteriological diagnosis, it is much better to use the liquid egg yolk medium than McCoy's medium. This applies not only to those cases where dead rodents are being investigated, but also to the diagnosis of diseased animals (even those very recently infected). The author feels that the liquid medium is better, because B. tularense proliferates starting from a smaller inoculation dose and that its growth rate is higher.

Table II

Pasteurella tularensis cultures obtained from dead animals by direct inoculation into the liquid medium. Tests on marmots subcutaneously infected with 5 million bacterial unit doses.

Time after infection	Inject. point		Lymph node		Spleen		Liver		Lung		Blood stream	
Killed after:	Liq. m.	McCoy's med.	Liq. m.	McCoy's med.	Liq. m.	McCoy's med.	Liq. m.	McCoy's med.	Liq. m.	McCoy's med.	Liq. m.	McCoy's med.
3 hours	+	+	+	-	+	-	+	-	-	-	-	-
1 day	+	+	+	-	+	-	+	-	-	-	-	-
2 days	+	-	+	-	+	-	-	-	-	-	-	-
3 days	+	-	+	+	+	+	+	+	-	-	-	-
4 days	+	-	+	+	+	+	+	-	-	-	-	-
Dead on 4th day	+	-	+	+	+	+	+	+	+	-	+	-

Symbols: + = bacterial growth; - = no bacterial growth.

Table III

Pasteurella tularensis cultures obtained from killed animals by direct inoculation into liquid medium. Experiments with house mice subcutaneously infected with single doses of 1,000 bacterial units.

Time after infection	Inject. point		Lymph node		Spleen		Liver		Lung		Blood stream	
	Liquid medium	McCoy's medium	Liquid medium	McCoy's medium	Liquid medium	McCoy's medium	Liquid medium	McCoy's medium	Liquid medium	McCoy's medium	Liquid medium	McCoy's medium
Killed after:												
5 hrs	+	-	-	-	-	-	-	-	-	-	-	-
1 day	+	-	+	-	+	-	-	-	-	-	-	-
2 days	+	+	+	+	+	-	-	-	-	-	-	-
3 days	+	+	+	+	+	+	+	+	+	-	+	-
4 days	+	+	+	+	+	+	+	+	+	-	+	+

Symbols: + = bacterial growth; - = no bacterial growth.

Pasteurella tularensis cultures obtained in the liquid medium from the bubonic punctate of experimental animals. It happens quite frequently in laboratory practice that animals employed in a biological experiment go through a long period of illness then die on the 8th - 10th day. On the other hand, many infected animals recover and it becomes impossible to isolate cultures of the tularemia agent in them. As a result, the bacteriological diagnosis of tularemia is considerably delayed.

In order to determine the possibilities of accelerating the growth of Pasteurella tularensis in cultures, the author adopted a method by which bubonic material was inoculated into the liquid medium. The group of experimental animals consisted of guinea pigs which had been infected with two Bact. tularense strains differing by their degree of virulence, either subcutaneously, or by rubbing the infectious material into the skin. All of these animals were periodically tested

by performing a puncture in the regional lymph gland and bacteriologically checking the fluid for the bubonic material. The first puncture was made 24 hours after the animals had been infected and thereafter every 24 hours. The material withdrawn from the regional lymph nodes was inoculated into liquid egg yolk medium and McCoy's medium. The pertinent results are presented in Table IV.

Table IV.

Tests with bubonic punctate from guinea pigs. Guinea pig No. 206, infected through the paw with strain No. 13.

Inoculated culture med.	Time elapsed since infecting injection								
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
Liquid egg yolk med.	+	+	+	+	+	+	Animal died		
McCoy's m.	-	-	-	-	+	+	Animal died		
Guinea pig No. 155, infected by rubbing strain No. 9 into skin.									
Liquid egg yolk med.	-	+	+	+	+	-	-	-	Animal survived
McCoy's m.	-	-	+	-	-	-	-	-	Animal survived

Symbols: + = bacterial growth; - = no bacterial growth.

In general one, or less frequently, two days after the animals had been infected it was possible to isolate Bact. tularense in the liquid egg yolk culture medium, while on McCoy's medium there was no proliferation unless the medium had been inoculated with material taken from an infected animal shortly before its death. Guinea pig No. 155 is a case deserving special attention. On the other liquid medium Bact. tularense could be isolated 2, 3, 4, and 5 days after the guinea pig had been infected, while on McCoy's medium, the bacterium could be isolated only once, namely 3 days after the infection. Guinea pig No. 155 survived the experiment and

remained under observation for the next six months.

This experiment was repeated a number of times proving each time that the isolation is considerably shortened if one inoculates the liquid egg yolk medium with bubonic punctate obtained from a biological test animal.

The liquid egg yolk medium helps accelerate the bacteriological diagnosis of tularemia during a natural epizootic outbreak of the disease. Her experimental studies have proved that the bacteriological diagnosis of tularemia in animals dead of, or diseased with tularemia can be more rapidly established with the aid of the liquid egg yolk medium, the author proceeded to test this medium under natural conditions, that is, during an epizootic outbreak of tularemia.

The rodents -- some of which were alive and some already dead -- were examined using such facilities as were offered by a simple field laboratory. The material on which the tests were performed consisted of specimens of such parenchymatous organs as the spleen and liver. They were cultured in the thermostat at 37°, after having been seeded both in the liquid egg yolk medium and in McCoy's medium.

The field laboratory had been supplied with 50 mice, 25 of which were sent to a nearby laboratory for study by the conventional methods. The other 25 mice, 19 of which were living and 6 dead, were sectioned and investigated at the field laboratory. Fourteen of them yielded material which on having been seeded in the liquid egg yolk medium, incubated for 12 hours in the thermostat, then transferred to McCoy's medium and cultured in the latter for another 24 hours, produced pure Bact. tularense cultures.

In this case, a definite diagnosis of tularemia could be established more rapidly (within 36 hours) by directly inoculating the liquid medium with organ material from the animals.

Meanwhile, at the nearby laboratory to which the other 25 animals had been sent, the first pure Bact. tularense cultures were obtained by the conventional biological methods only after 9 days.

The liquid culture medium was tested the same year a second time, during a summer outbreak of tularemia. On this occasion, the laboratory received 6 water rats from the suspected area. Five of these rats were alive, while one was dead. Pure Bact. tularense cultures were isolated in all of them in 36 hours in all 5 cases. Only two of

the animals yielded material which produced cultures in McCoy's medium.

Shortly thereafter, a widespread epizootic outbreak having occurred among the blind rat-moles of a certain area, a field trip was organized with the purpose of determining the nature of the disease in question. The methods employed consisted of the conventional techniques, as well as of culturing organ material in the new liquid egg yolk medium.

The total number of animals investigated was 260. Eighteen bacterial strains were obtained from liquid medium cultures seeded with organ material from rat-moles, hamsters and marmots.

Not a single culture was obtained by the conventional methods, probably as a result of the fact that the expedition lacked the time and facilities to repassage the material in laboratory animals.

The first observations made on different types of rodents in natural tularemia foci during spontaneous epizootic outbreaks of the disease, with reference to the use of the liquid egg yolk medium as a means of accelerating the bacteriological diagnosis, have fully confirmed the laboratory findings.

Conclusions.

1. Isolation of pure tularemia cultures is materially accelerated by directly inoculating organ material from animals dead of, or infected with tularemia into the liquid egg yolk medium.

2. The bacteriological diagnosis of tularemia can be established more rapidly by inoculating the liquid egg yolk medium with bubonic punctate from biological test animals. In her own experiments, the author was able to isolate Bact. tularense cultures by this method already 1 or 2 days after the animal had been infected.

3. During a natural epidemiological outbreak of tularemia caused by animals, use of the liquid egg yolk medium simplified and considerably accelerated the diagnosis of tularemia by permitting to isolated pure cultures of Bact. tularense without animal passage by simple, direct inoculation with animal organ fragments.

4. On the strength of the laboratory findings and practical results obtained during a natural epizootic outbreak of the disease, the liquid egg yolk culture medium is recommended as a means which can help establish a rapid bacteriological diagnosis of tularemia in rodents.

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