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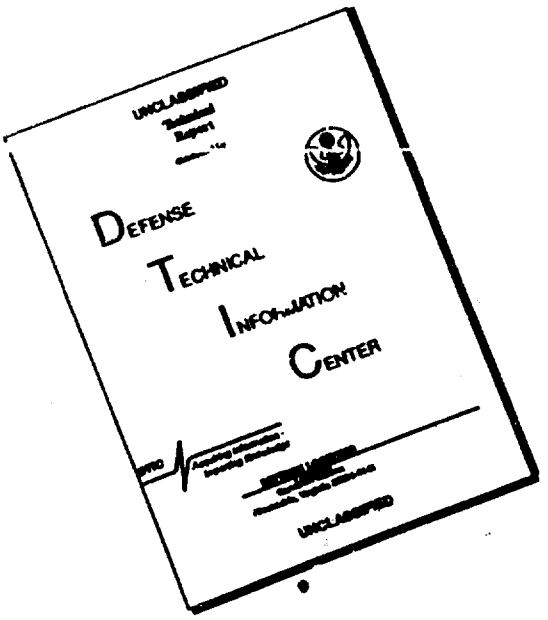


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# THE ISOLATION OF THE BRUCELLOSIS PATHOGEN FROM THE EYES OF GUINEA PIGS FOLLOWING EXTRAOCULAR INFECTION

/Following is a translation of an article by V.M. Kiseleva, L.G. Lopatukhina, and F.I. Usmanova of the Kazakh Medical Institute, the Central Asian Anti Plague Institute, and the Kazakh Scientific Research Veterinary Institute in the Russian-language periodical Zhurnal mikrobiologii, epidemiologii, i immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), No 7, 1963, pages 120-125. The article was submitted on 10 November 1961./

Despite the very extensive Soviet literature on brucellosis, no attention has been devoted to a bacteriological investigation of the eyes in the case of this infection. It must be noted that foreign science has also not produced any concrete information on this definitely important problem. In connection with this we felt that it was expedient to study the frequency of the isolation of Brucella organisms from the eyes of guinea pigs. We studied its relation to the infecting dose and the virulence of the infecting strain, to the periods of time from the moment of infection, to the presence in the animal of an experimental endogenous brucellosis infection of the eyes, and to enteral, intranasal, and subcutaneous vaccination which preceded the infection of the animals as well as to the periods from the moment of vaccination to infection.

Table 1

Results of a bacteriological and serological investigation of guinea pigs one month after subcutaneous infection with various doses of Br. Melitensis No. 487

| Number of infected animals | Number of microbial cells in the infecting dose | Number of animals | | | | Number of isolated cultures of Brucella organisms | | | Number of animals with positive discharges from the eyes | Number of eyes with positive discharges | Wright's reaction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | With developed infection | | | With undeveloped infection | From the organs | From the blood | From the eyes | | | 1:800 | 1:400 | 1:200 | 1:100 | Negative | Not investigated |
| | | Generalized | Glandular | Regional | | | | | | | | | | | | |
| 10 | 1000 | 10 | - | - | - | 69 | 7 | 17 | 8 | 11 | 8 | 1 | 1 | -[1] | - | - |
| 10 | 100 | 7 | 1 | - | 2 | 53 | 1 | 11 | 7 | 9 | 2 | 2 | 1 | 2[1] | 1 | 2 |
| 5 | 50 | 8 | - | - | - | 59 | - | 9 | 5 | 7 | - | 3 | 5 | - | - | - |

[1] Of them there was one guinea pig with undeveloped infection.

Table 2

Results of a bacteriological and serological investigation of guinea pigs one month after subcutaneous infection with a series of virulent cultures of Brucella organisms of the cattle and sheep types (100, 1000, 250,000, 500,000, 1 million, and 1 billion microbial cells.

| Number of infected animals | Number of the infecting strains | Number of animals: With developed infection: Generalized | Glandular | Regional | With undeveloped infection | Number of isolated cultures of Brucella organism: From the organs | From the blood | From the eyes | Number of animals with positive discharges from the eyes | Number of eyes with positive discharges | Wright's reaction: 1:1280 | 1:640 | 1:320 | 1:160 | 1:80 | 1:40 | 1:20 | 1:10 | Negative | Not investigated |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 487 | 11 | - | 1 | - | 79 | 2 | 10 | 9 | 13 | - | - | 1 | 1 | 2 | 4 | 1 | 3 | - | - |
| 12 | 200 | 11 | - | - | 1 | 72 | 3 | 16 | 9 | 14 | - | - | 3 | 2[1] | 3 | 2 | - | - | - | - |
| 11 | 4004 | 10 | - | - | 1 | 58 | 2 | 14 | 9 | 12 | - | 4 | 2 | 2 | 1 | - | - | - | 1 | 1 |
| 12 | 3/9 | 11 | 1 | - | - | 68 | 1 | 11 | 7 | 9 | - | 3 | - | 2 | 3 | 1 | 3 | - | - | - |
| 12 | Stellate | 11 | - | - | 1 | 53 | 1 | 7 | 4 | 5 | 1 | - | 1 | 1 | 1 | 3 | 1 | 2 | 1 | 1 |

[1] Of them there was one guinea pig with undeveloped infection.

We received approximate data on the inoculation of Brucella organisms from the tissue and fluids of the eyes of some animals in 1956 (Kiselev and Usmanov, 1957). In 1959, material was published which described the relation of the frequency of the isolation of Brucella organisms from the eyes of guinea pigs to the antigenic load and preliminary vaccination (Kiseleva, Lopatukhina, and Usmanova). Further numerous investigations in this direction, while confirming the effect of the infecting dose (Table 1) and the virulence of the infecting strain (Table 2) on the frequency of positive secretions from the eyes, at the same time showed that a month after subcutaneous infection with virulent cultures of Brucella organisms of the cattle and sheep types in minimum infecting doses of 5-10 microbial cells it was possible to isolate Brucella organisms from the eyes of all the test animals. The same kind of results was obtained for some animals a month after subcutaneous infection with 50 microbial cells; however, the number of eyes which were infected with Brucella organisms was less.

In tests (See Table 2) it was noted that of 2 animals which had been infected with the same dose, a brucellosis culture was secreted from the eyes of one animal but not from the eyes of the second. The data which was obtained also indicated that a generalized infection did not always develop evenly in all animals infected with the same dose. In some test animals only the lymph nodes or one regional lymph node were infected with Brucella organisms; otherwise the animal might remain free of Brucella organisms but might change immunologically; or, finally, neither the appearance of Brucella organisms in the organs and and lymphatic system nor signs of immunological rearrangement might occur. Consequently, depending on the individual differences in some test animals, contamination of the eyes with Brucella organisms could be detected one month after infection; in others it could not be detected.

In order to determine the effect of the time from the moment of subcutaneous infection of guinea pigs on the frequency of the discharge of Brucella from the eyes, we conducted a bacteriological investigation of the conjunctival secretions, tissue, and fluids of the eyes at various times. In the conjunctival secretions of guinea pigs we detected Brucella organisms within 7, 14, 21, 24, 26, 35, 43, 52, and 66 days after subcutaneous infection with virulent Brucella cultures of the cattle and sheep types.

In all, 45 guinea pigs were investigated; in 22 of them we were able to isolate Brucella organisms from the conjunctival secretions (in 18 cases from one eye and in 4 cases from both eyes).

It was possible to isolate Brucella organisms from the conjunctival secretions of animals with brucellosis infections of the eyes as well as from animals without such infection. This number included one guinea pig with unilateral brucellosis keratoiridicyclite, Brucella organisms were isolated from the conjunctival secretion of both eyes after 52 days and for a second time 66 days after infection. Consequently, the positive bacteriological findings in the conjunctival secretion were not connected with brucellosis infection in the eye but rather depended on the total generalization of the brucellosis pathogen in the organism of the guinea pig. Cases were also noted where brucellosis organisms were isolated from conjunctival secretion within a month after subcutaneous infection but not from the tissue and fluids of the eye, and vice versa. Such discrepancies were noted in 8 of 14 cases.

Brucella organisms were both found simultaneously in the conjunctival secretion and in the tissue of the eye or were simultaneously absent in them.

Thus, it can be assumed that bacteriological investigation of conjunctival secretion merits wide usage as one of the components of the complex method of laboratory diagnosis of brucellosis infection during the early stages of this disease in man and animals.

In the great majority of cases the brucellosis pathogen remained rather briefly in the tissue and fluids of the eye (Table 5). The periods spent by the Brucella organisms in the eye depended not only on the intensity of the antigenic load but also on the individual properties of the test organism.

In animals with experimental endogenous brucellosis diseases, the isolation of Brucella organisms from the eyes was possible during the early periods after the infection of the animal and the beginning of the inflammatory process in the eye. The etiological role of reproduced endogenous brucellosis keratoiridocyclites was definitely shown by the positive bacteriological findings obtained during an investigation of 7 eyes (Kiseleva,

1960). In cases in which the pathogen was not isolated its absence can be explained by the fact that the pathological process in the eye continued for a longer time than the live pathogen remained in the tissue of the eye.

Table 3

The results of a bacteriological investigation of the tissue and fluids of the eyes of guinea pigs at various times after subcutaneous infection with Br. melitensis No 487 and Br. abortus bovis No 4004

| Number of guinea pigs | Infecting dose (number of microbial cells) | Time after infection (in months) | | | | Number of guinea pigs with positive discharges from the eyes | Number of infected eyes |
|---|---|---|---|---|---|---|---|
| 72 | 50 | 2½ | 3 | 3½ | 4 | - | - |
| 16 | 1 million | - | 3 | - | - | - | - |
| 12 | 1 billion | - | - | 3½ | - | 4 | 8 |

In as much as at the present time the vaccination of people (Vershilova, Ferer, and Polyakova, 1954; Kaytmazova, 1954) and animals (Studentsov, 1961) on farms which are unfavorable with respect to brucellosis is one of the basic scientifically supported an active means of preventing this disease, we studied the effect of prophylactic vaccination on the discharge of Brucella organisms from the tissue and fluids of the eyes as related to certain methods and periods of inoculating Br. abortus bovis 19 prior to experimental infection.

Table 4

[illegible] of the isolation of Brucella organisms from the eyes of guinea pigs in relation to the [illegible] of the [illegible] preceded the infection

| Test series | Method of vaccination (in microbial cells) | Vaccine dose (number of microbial cells) | Time from the moment of vaccination to the moment of infection (in days | Number of the infecting strain and method of infection | Number of guinea pigs: In the test — In each group | In the test — Total | Not infected — In each group | Not infected — Total | Infected — In each group | Infected — Total | Of them by force — Regional | Of them by force — Glandular | Of them by force — Generalized | With positive discharges from the eyes — In each group | With positive discharges from the eyes — Total | Number of eyes with positive discharges — In each group | Number of eyes with positive discharges — Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | Peroral. . . | 10 Bil. | 30 | Br. melit. No 487, 50 microbial cells subcutaneously | 9 | 36 | 4 | 13 | 5 | 23 | – | – | 5 | 1 | 2 | 2 | 3 |
| | Intranasal . " . | 100 Mil. 1 Bil. | | | 10 | | 4 | | 6 | | 1 | – | 5 | 1 | | 1 | |
| | Subcutaneous | 1 Bil. | | | 7 | | 2 | | 5 | | – | 1 | 4 | – | | – | |
| | Unvaccinated | | | | 10 | | 3 | | 7 | | – | 2 | 5 | – | | – | |
| | | | | | 10 | | – | | 10 | | – | – | 10 | 10 | | 16 | |
| II | Peroral. . . | 10 Bil. | 60 | Ditto | 9 | 36 | 2 | 10 | 7 | 26 | – | 1 | 6 | – | 1 | – | 2 |
| | Intranasal . " . | 100 Mil. 1 Bil. | | | 9 | | 2 | | 7 | | – | – | 7 | – | | – | |
| | Subcutaneous | 1 Bil. | | | 9 | | 3 | | 6 | | 2 | – | 4 | 1 | | 2 | |
| | Unvaccinated | | | | 9 | | 3 | | 6 | | – | 1 | 5 | – | | – | |
| | | | | | 9 | | – | | 9 | | – | – | 9 | 9 | | 14 | |
| III | Peroral. . . | 10 Bil. | 90 | " " | 10 | 39 | 4 | 6 | 6 | 16 | – | – | 6 | – | 2 | – | 2 |
| | Intranasal . " . | 100 Mil. 1 Bil. | | | 10 | | 5 | 23 | 5 | | – | 3 | 2 | 1 | | 1 | |
| | Subcutaneous | 1 Bil. | | | 10 | | 6 | | 4 | | – | – | 4 | 1 | | 1 | |
| | Unvaccinated | | | | 9 | | 8 | | 1 | | – | – | 1 | – | | – | |
| | | | | | 10 | | 1 | | 9 | | – | – | 9 | 5 | | 8 | |
| IV | Peroral. . . | 10 Bil. | 180 | " " | 8 | 39 | 2 | 21 | 6 | 18 | 1 | – | 5 | 2 | 7 | 2 | 8 |
| | Intranasal . " . | 100 Mil. 1 Bil. | | | 13 | | 10 | | 3 | | – | – | 3 | 2 | | 3 | |
| | Subcutaneous | 1 Bil. | | | 9 | | 3 | | 6 | | 3 | – | 3 | 3 | | 3 | |
| | Unvaccinated | | | | 9 | | 6 | | 3 | | – | 3 | – | – | | – | |
| | | | | | 10 | | – | | 10 | | – | – | 10 | 8 | | 13 | |

Of the three tested methods of vaccination (subcutaneous, peroral, and intranasal), the subcutaneous method was the most promising for the eyes; the other two methods did not provide inoculative immunity in all cases (Table 4). Unfortunately, the effectiveness of the skin method was not investigated.

## Conclusions

1. The frequency of the isolation of Brucella cultures from the tissue and fluids of the eyes of guinea pigs a month after subcutaneous infection with virulent Brucella cultures depends not only on the intensity of the antigenic load but also on the individual properties of the test animals. When using minimum infecting doses all or almost all the eyes of test guinea pigs can be contaminated with Brucella organisms.

2. The bacteriological investigation of conjunctival secretion merits wide usage in the complex laboratory diagnosis of brucellosis infection in the early stages after the infection of man and animals.

3. The times of the discharge of Brucella organisms from the tissue and fluids of the eyes after the subcutaneous infection of guinea pigs are small and depend on the antigenic load and the individual properties of the test organisms.

4. It is possible to isolate Brucella organisms from the eyes with experimental endogenous brucellosis ailments of the keratoiridocyclite type by means of culturing in artificial nutrient media and infecting the animals; in this, as the time from the beginning of the inflammatory process in the eye and from the moment of infection increases, this possibility is lessened.

5. The formation and duration of the resistance of the eye tissue to Brucella contamination are more reliably provided by subcutaneous vaccination with Brucella organisms of strain Br. abortus bovis 19 than by enteral and intranasal vaccination with the same strain.

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