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AD835142
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DD83514Z

TRANSLATION NO. 1549

DATE: 5 November 1968

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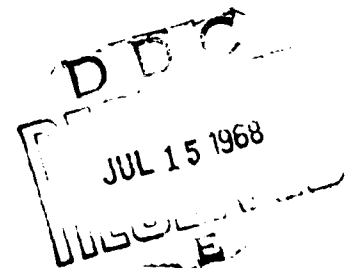
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THE INCIDENCE OF SPECIFIC NEUTRALIZING ANTIBODIES IN THE  
BLOOD OF INDIVIDUALS EXPOSED TO FOOT-AND-MOUTH VIRUS

Lucrarile Stiintifice ale  
Institutului de Seruri si  
Vaccinuri Pasteur (Scien-  
tific Studies of the Pas-  
teur Institute of Serums  
and Vaccines) No 7, Bucha-  
rest, 1963, pages 79-83

M. Popa et al

In specialized publications the first recorded cases of the exposure of human beings to the foot-and-mouth virus date from the eighteenth century, when the disease was diagnosed by monks who drank milk from diseased cattle(8).

Since then several writers have published clinical studies on the incidence of the disease in human beings, diagnosed by its general symptoms (fever, loss of appetite, headiness [“cefalee”], painful joints, etc.), and its local symptoms (the appearance of aphthae on the gingival-labial mucous membrane, on the tongue, on the palm, on the sole of the foot, etc.). A great many such cases have been described, especially among persons who had come in contact with diseased animals. In 1895 alone Siegel and Bussensis (see 3) published 1500 case histories, including some of fatal cases. For many years the diagnosing of the foot-and-mouth virus was based upon anamnesis and clinical symptoms, not always reliable criteria considering the fact that the lesions thought to be pathogenic can be confused with those of herpes simplex, multiform exuding erythema--the vesicular form, pemphigus, unspecified vesicular stomatitis, variola, common staphylococcus infections, etc. In the light of these findings, most of the human cases attributed directly infection by the foot-and-mouth virus are contestable. Self-inoculations [See Note], likewise numerous, can also be contested unless they succeed in

[Note]: The first self-inoculation in the history of this human-animal disease was conducted in 1835 by Mertwig, Mann and Vullain, who daily drank one-fourth of a liter of milk from sick cattle. All three writers experienced eruptions on the buccal mucous membrane, the gums, the palms and other severe symptoms. They did not re-infect the respective animals.

re-infecting the respective animal with infectious material taken solely from human lesions (aphthae, lymph) or from the blood, urine, faeces and other pathological materials which can contain the virus in its virulent or post-virulent phase (1, 2, 5, 9).

Some writers succeeded in re-infecting their experimental animals, such as guinea pigs (Nicolle, Pancera, Magnuson, Hermanson, etc.) or young calves (Schautze, Bertarelli, Favero) or young sows Schneider).

A further proof offered by the writers who have achieved this transmission is that the serum from the men who were infected by the disease either naturally or experimentally, extracted from 10 to 15 days after the appearance of their symptoms, sometimes succeeded--in doses of 0.4 to 0.6 to 2 milliliters per guinea pig--in immunizing the animal against extensive aphthae (6, 9).

Our work did not deal with apparent infection of the foot-and-mouth virus but only with its "hidden" infection. In fact, as with other inframicro-organic diseases, it is thought that the percentage of those having the disease but not exhibiting its symptoms is far greater than that of those with the clinical symptoms. Until now there has been no survey of the relative proportions of the clinical forms and the "hidden" forms of the human diseases which accompany the epizooty of foot-and-mouth disease. There are also very few basic studies of the "hidden" diseases based on the titration of complementary fixing antibodies and serum neutralizers. RFC ("Ratia Fixatorilor de Complement"?; Fixing Complement Ratio?) was used to discover the specific antibodies in the serum of various persons who took part in the preparation of anti-aphthous vaccines (the conditions in general approximated those which we have described) without precise results or with results which were not in accordance with the intensity of the contact with the virulent material.

RFC yields inconclusive results because of the numerous unspecified reactions of persons suffering from influenza, infectious hepatitis or other inframicro-organic diseases. The serum neutralization of guinea pigs is another method incapable of indicating the presence of small quantities of antibodies generated during the course of "hidden" infections (4, 7, 9).

Vetterlein (see 6) thinks that the most effective method of indicating the presence of the immunizing antibodies acquired by persons in the course of "hidden" infections is the serum immunization of suckling mice.

Using this method Vatterlein [sic] tried to titrate the level of neutralizing antibodies in the blood of 34 persons who worked with the virus. His results differ from ours. We could show the presence of antibodies only in five persons with a percentage of 15 percent positive subjects (6, 9).

#### OUR EXPERIMENTS

In our experiments we tested serums extracted from personnel from diverse work areas within the section which deals with inoculation and with

extracting the virulent material necessary for preparing the anti-aphthous vaccine.

Within this section the personnel performed various tasks some of which (inoculation, extracting) were conducted in a highly infectious environment. This was done through the direct handling of aphthous antigens, and of the ingual epithelium of experimental cattle infected with the virus. Another class of workers came in contact with the virus to a more limited extent, doing transporting work, receiving animals, disinfecting, guarding, etc. Hereafter the first group will be designated CI ("contact intense"; intense contract), while the second group will be designated CS ("contact slight"; slight contact).

At the same time experiments were conducted on serums obtained from persons who did not come into contact with the virus at all; these served as a control group and were designated FC ("fara contact"; no contact).

#### PROCEDURE

The serums obtained were inactivated placed in cold storage (+4 to +6 degrees C) until a reaction occurred. Tests of the serums were conducted from 15 to 75 days after extraction.

In order to decelerate and titrate the specific antibodies we employed the serum neutralization method on suckling mice from 6 to 8 days old. The antigen used for the test consisted of a suspension of muscle tissue from suckling mice who had died from aphthous virus infection with a titer of between  $10^{-7}$  and  $10^{-9}$ .

Fresh antigen, prepared each time, was used for each serum neutralization test. The suspension was made using phosphate pH = 7.6 as a dilutant.

The serums employed were extracted from the persons concerned.

The antigen was the variable factor, the serum remaining constant. The dilutions of the antigen were prepared within the limits  $10^{-1}$  through  $10^{-9}$  inclusive. The various dilutions of the virus were placed in contact in vitro with equal quantities of serum for 1 hour at 26 degrees C. Dilutions of the virus with which the control group was inoculated were maintained under the same conditions. Generally two mice were inoculated with 0.2 milliliters each of every one of the sample serum neutralizers--in turn--subcutaneously in the dorsal region and with 0.1 milliliters each of the control virus in the same region, subcutaneously. Throughout the testing those in the control group were inoculated with the weakest dilutions ( $10^{-6}$  through  $10^{-10}$  inclusive) in order to specify exactly the titer used in each test.

The mice were observed for 10 days. In general those in the control group died in the first 48 hours, while the mice inoculated with the trial serums and those which had not developed an immunity died by the seventh or

eighth day after inoculation, according to the quantity of antibodies contained in the respective trial serum and to the resistance of the individual animal.

Altogether thus far 38 trial serums have been studied, 28 of which were provided by persons working in the infected environment and 10 by persons not exposed to the aphthous virus.

Of the 28 persons who worked in the infected environment, 22 performed their duties in a highly infected environment (the CI group) and 6 in the slightly infected environment (the CS group).

#### RESULTS AND THEIR INTERPRETATION

Since the immunization value of the serums studied was quite uneven, in order to facilitate interpreting the data obtained the results were divided into three categories: positive, inconclusive and negative.

The positive serums were in turn subdivided into:

1. Highly positive (+++) which neutralized the dilutions containing the largest quantities of virus ( $10^{-2}$  through  $10^{-3}$ );
2. Positive (++) which neutralized dilutions containing smaller quantities of virus ( $10^{-4}$  through  $10^{-6}$ );
3. Weak (+) which neutralized dilutions containing very small quantities of virus ( $10^{-7}$  through  $10^{-8}$ ).

All of the positive serums, in reacting with the serum neutralization, protected the inoculated mice until the second dilution.

This subdivision of the positive serums corresponds to the estimates which were derived from the reaction of the serum neutralization of titer in the immunizing antibodies of the serums obtained from cattle which had been vaccinated or which had survived the disease, in order to test the potency of the immunization.

In the second category are the inconclusive serums which protected the mice through only one dilution in the serum neutralization reaction, their resistance being attributable to other factors.

The last category contains the negative serums, those which in the serum neutralization reaction did not protect a single mouse through any of the dilutions. The results obtained have been compiled in Table 1.

A study of the data in Table 1 shows that of the 22 trial serums extracted from persons who came in intense contact (CI) with the infected environment, 54.5 percent are positive and 45.4 percent highly positive. With the FC group (no contact) there were no positive serums.

Table 1

## Results Obtained in Serum Neutralization

Type of contact	No. of tests	Results obtained							
		Positive				Inconclusive		Negative	
		+++	++	+	% total	Nr.	% total	Nr.	% total
Intense (C.I.)	22	5	5	2	54.5	1	4.6	9	40.9
Weak (C.S.)	6	1	-	1	33	-	-	4	67
No contact (F.C)	10	-	-	-	0	2	20	8	80

Legend: +++ - Neutralizes virus dilutions from  $10^{-2}$  to  $10^{-3}$  inclusive;  
 ++ - neutralizes virus dilutions from  $10^{-4}$  to  $10^{-6}$  inclusive;  
 + - neutralizes virus dilutions from  $10^{-7}$  to  $10^{-8}$  inclusive.

The two inconclusive serums in the FC category protected the suckling mice against the foot-and-mouth virus only in the later, very weak dilutions ( $10^{-7}$  through  $10^{-8}$ ), which does not rule out either the presence of another disease being in the persons from which the respective samples were taken--one which might have contained unspecified antibodies--or the presence of various factors which might have influenced the suckling mice used in the experiment.

Among the workers who lived in the infected environment three cases of vesicular stomatitis were noted which we considered to be aphthous and which developed without general symptoms. In the serum neutralization test upon the mice all of these specimens gave negative results, while our attempt to isolate the aphthous virus in the tryptic cells of cattle, as well as in suckling mice from the lymph obtained from the vesicles of some of them which were diseased proved unsuccessful.

Regarding the duration of the antibodies in human beings, we have made only a beginning through the titration of two serums which are highly positive from a first estimate. It has been determined that the serum neutralizing titer is maintained at the same level even 14 months after contact with the aphthous virus.

In order to draw a definitive conclusion, continual systematic experiments concerning the duration of antibodies in the blood of persons who are no longer in contact with the virus are required.

In conclusion, we may state that in the serum of persons who have come into contact with the aphthous virus there appear specific neutralizing antibodies of the  $O_2$  type. The titer of the neutralizing antibodies is directly proportional first to the intensity of the contact and second to the reactivity of the individual.

Our experiments have also shown that following intense and prolonged exposure (with some persons about 2 months) the percentage of positive subjects reaches 54.5 percent, which proves that man has a relatively low susceptibility to aphthous infection. In support of this view is the fact that this disease among human beings generally takes the "hidden" form; there are very few clinical cases, and they are often contestable.

Regarding the duration of antibodies in man, it appears that the serum neutralizing titer is maintained at the same level for a prolonged period of time (more than a year) after contact with the aphthous virus has ended.

#### CONCLUSION

In the course of the experiments concerning the frequency of neutralizing antibodies in the blood of various persons who had come in contact with the foot-and-mouth virus 38 trial serums were tested, of which 28 were provided by persons who had worked in an infected environment and 10 were provided by persons who had not come in contact with the foot-and-mouth virus, these last subjects serving as a control group.

The presence of neutralizing antibodies was established by the serum neutralization method on suckling mice 6 to 8 days old.

1. The data obtained show that there are specific neutralizing antibodies in the blood of persons who have been exposed to foot-and-mouth disease.

2. Among persons working in an infected environment, 54.5 percent were positive subjects while there were no neutralizing antibodies in the persons who had not been exposed to the virus.

3. In the cases studied the disease always took a "hidden" form; that three persons contracted vesicular stomatitis can not be attributed to the foot-and-mouth virus since the virus could not be isolated from the lymph extracted from the vesicles of these persons.

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