

The Use of Live Vaccine for Vaccination of Human Beings against Brucellosis in the USSR

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The great majority of human brucellosis cases in the USSR are caused by contact with infected sheep and goats. Extensive action has been taken to prevent human infection and to reduce the incidence among farm animals, the main prophylactic measure in recent years being vaccination with live brucellosis vaccine. The author summarizes the steps leading to the development of a satisfactory vaccine and gives a brief description of the method of preparation. Discussing the results obtained, she states that there has been a nearly 60% reduction in the number of human cases over the period 1952-58.

The subcutaneous route of administration is usually resorted to, but preliminary figures suggest that cutaneous vaccination is equally effective immunogenically, although in persons who have suffered from active brucellosis it causes strong reactions and may lead to exacerbation of the disease. Research is going forward into the development of a cutaneous vaccine capable of general use.

INTRODUCTION

Epizootiological and epidemiological data now available show that brucellosis is distributed quite widely in many countries. In the USSR brucellosis of all three types is encountered, but the bovine and porcine types rarely cause human illness (0.3%-3.6%). It is brucellosis of goats and sheep which is most important in the Soviet Union. Its area of distribution covers the districts where sheep-rearing is widely practised, and particularly where pedigree fine-fleeced sheep are raised. Sheep and goats are the source of infection in 85%-95% of cases of illness accompanied by clinical symptoms of the disease both among people engaged in livestock rearing and among the population in contact with sick animals. In the processing of raw material and food products (meat) from brucellosis-infected sheep and goats, the number of cases of illness fluctuates between 5%-8% and 14%-20%. The transmission of the disease in food has been reduced by the introduction of hygienic measures governing the processing of dairy products.

In the Soviet Union extensive action has been taken to eliminate brucellosis among farm animals

and at the same time measures of prophylaxis have been organized to prevent human infection. Since 1953-54, wherever there is a risk of human infection with brucellosis of goats and sheep, the main prophylactic measure taken has been the vaccination of human beings with live brucellosis vaccine.

Despite the continuing unfavourableness of the epizootiological situation among sheep and goats in a number of districts, the incidence of brucellosis has been reduced annually during the past seven years. In the USSR as a whole the incidence of brucellosis during the period 1952-58 has fallen by 59.5%. Thus the epidemiological figures for brucellosis of late years have pointed the way to practical measures of prophylaxis against the disease through vaccination with live vaccine.

It is well known that the use of vaccine for brucellosis prophylaxis was a failure for many years, and it was only the knowledge gained with regard to pathogenesis and immunity which made it possible to produce effective vaccine. The main theoretical premises on which human vaccination with live vaccine made from strains of the bovine type as a protection against brucellosis of sheep and goats was based were as follows:

(a) the existence in brucellosis infection not only of infection immunity but also of post-infection sterile immunity;

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(b) the possibility of establishing cross-immunity to variants of *Brucella*, i.e., the existence in brucellosis of a non-type-specific immunity;

(c) the establishment of the fact that the vaccinal process caused by various vaccine strains of the bovine type (19, 104-M, L₁, L₂) follows a benign and short course;

(d) the relationship which was proved between the solidity and duration of immunity and the intensity of the vaccinal process; thus, the more intensive and long-lasting the infection of the organism by the vaccinal culture (this being dependent on its residual virulence and the dose), and the more marked the reaction in the reticulo-endothelial system and the more marked the immunological shift, the more complete the resistance built up to a virulent culture of *Brucella melitensis*.

A considerable amount of evidence has also accumulated in epidemiological practice to prove that persons who have been infected with bovine or porcine brucellosis possess good immunity against brucellosis contracted from sheep and goats.

DEVELOPMENT OF BRUCELLOSIS VACCINE

When we studied the immunogenic effectiveness of various strains of low virulence (*Br. melitensis* 2, *Br. suis* 22, *Br. abortus* 98, 19) on guinea-pigs, white mice and sheep, we found the most effective vaccinal strain to be *Br. abortus* 19 (Buck Cotton) (Vershilova & Shtriter, 1937; Voskresenski, 1937; Vershilova, 1947a, 1947b). The variant of this strain available in our laboratory was labile. When cultures were seeded on agar, dissociated colonies were formed. By selection, a suitable population of *Br. abortus* culture was obtained, called 19-BA by Vershilova to distinguish it from the original strain. By experimental research we determined the immunogenic dose of the 19-BA culture on subcutaneous and cutaneous administration to guinea-pigs, and studied the vaccinal process and the solidity and duration of immunity in the non-sterile and sterile phases to a virulent culture of *Br. melitensis* and *Br. suis*.

The first test of the innocuity of the 19-BA vaccinal strain on subcutaneous administration in a dose of 20-40 million brucellae was carried out on volunteers in 1946. Later, in 1947-52, a dried vaccine was made from the *Br. abortus* strain 19-BA, and was used to vaccinate 5000 workers tending brucellosis-infected sheep or coming into contact in their work with raw material from such sheep. Five years' research on this vaccine (Vershilova,

Feder & Polyakova, 1952) established that an immunogenic and harmless vaccine dose in subcutaneous vaccination amounted to 300-600 million brucellae and demonstrated that the vaccine caused little reaction and was harmless. Local reaction on injecting the vaccine into the shoulder or the subscapular region took the form of hyperaemia, with an area of infiltration measuring 1-6 cm. The infiltration was maintained for 5-6 days, and in individual cases, longer, falling in size to that of a pea. A marked reaction, 2-4 cm, was recorded in 47% of cases. In the rest, reaction was either slight or absent. In individual cases the reaction was very marked. This occurred in persons who had suffered from brucellosis in the past, or who had been in long contact with sick animals or the products obtained from them. It was established that these reactions to vaccination were caused by specific sensitization, which cannot be demonstrated by skin testing with 0.1 ml of brucellin. In the majority of those vaccinated there was no manifestation of a general reaction. Some persons (8%) complained of general malaise and headache. A rise in temperature from 37.2°C to 37.6°C for 24-48 hours was observed rarely (2%). We failed to establish a relationship between the strength of the reaction to vaccination and the size of the dose of vaccine we were testing. We paid special attention to study of the innocuity of the live vaccine. Clinical observations of vaccinated persons were carried out by clinicians (Aslanov, Vasilyeva, Feder, Piletskaya, Lukasheva, and others) with long experience in brucellosis work. The vaccinated persons were questioned closely and examined carefully and their temperatures taken for 1½-2 months. During these observations no clinical signs of brucellosis were found in the persons vaccinated. There were also no haematological changes, except for slight fluctuation in white blood cells. Subsequent observations after mass vaccination of persons aged from 10 to 70 years also confirmed the innocuity of the live vaccine made from *Br. abortus* strain 19-BA.

The immunological reactions clearly depict the organisms' response to the influence of the live vaccine, as has been shown by our observations and those of a number of other authors. It was found that the immunological reactions were maintained for a long time (for 2-3 years in 56%-63%) in the majority of persons working in foci of brucellosis or in abattoir slaughtering-halls and gutteries. The serological reactions appear early. The change in the allergic pattern of the organism occurs later.

Kaitmazova (1954) established that the vaccinations cause a symptomless vaccinal process to develop in the organism, with the vaccine strain sometimes being detected in the blood. Of 161 blood cultures made 7, 15 and 30 days after vaccination, the vaccine strain was cultured in one case after 15 days, although there were no symptoms of brucellosis of any kind in the person concerned.

An indication of the effectiveness of the vaccination is given by comparison of the incidence of fresh brucellosis among unvaccinated and vaccinated persons in the farms where vaccination was carried out. Our original five years' observation of persons vaccinated with the live brucellosis vaccine proved its high prophylactic effectiveness: among those vaccinated during those years, an average of 0.5% contracted the disease an appreciable time after vaccination, whereas the figure among the unvaccinated in the same group was 12.3%.

These data served as a basis for the extensive vaccination of human beings in foci of brucellosis of sheep and goats. The data we collected (Vershilova & Golubeva, 1956) in evaluating the effectiveness of vaccination of 200 000 workers and other persons on livestock farms where the epizootiological situation was very unfavourable showed a reduction in the incidence of the disease in the vaccinated persons of 3.3 times, and in one case of as much as 11.2 times. Vaccination of workers in the meat industry occupies an important place in brucellosis prophylaxis. Prophylactic vaccination among this group of workers led to a reduction in the incidence of brucellosis to 0.4%-3% and even, in some instances, to complete disappearance of the disease in many enterprises where vaccination of newly engaged workers was introduced (Drankin, 1956; Lkhov, 1957).

The vaccine used was prepared in large quantities according to a method developed by the Brucellosis Laboratory of the Gamaleya Institute of Epidemiology and Microbiology. This method may be summarized as follows:

After 36-48 hours' incubation on liver or potato agar the vaccine culture is washed down with a stabilizer medium (10% saccharose, 1% gelatin and distilled water). The required standard of microbial suspension in respect of the content of brucellae in 1 ml is established and dispensed into ampoules (0.2 ml in each) and then dried. The density of the microbial suspension is determined on the basis of the number of inoculation doses required in one ampoule. One inoculation dose of brucellosis vaccine for subcutaneous injection should contain 400-600 million viable brucellae.

Apparatus of any design based on the sublimation principle is used for drying the living vaccine. The vaccine frozen at -30°C is dried under a vacuum of at least 100 microns. The temperature in the final stages of drying should be not more than $+25^{\circ}\text{C}$. The duration of drying is so arranged as to ensure that the residual humidity of the dried product is between 1.5% and 3%. The ampoules containing dried vaccine are sealed under vacuum. Each batch of prepared vaccine is bacteriologically tested for purity, innocuity on injection into white mice and the viability of the brucellae in the vaccine, determined from the number of colonies grown from a subculture of the vaccine. One in every ten batches of the vaccine is tested for immunogenicity by vaccinating guinea-pigs with 1 000 000 000 brucellae. After 20-30 days these guinea-pigs are infected with two infective doses of a virulent strain of *Br. melitensis* and after 30 days they are dissected for bacteriological examination. The vaccine is passed for issue if it is free from adventitious microflora and is harmless, if at least 70% of the brucellae have remained viable and if it protects 70% of the guinea-pigs from infection.

An analysis of the figures for the incidence of the *melitensis* infection among those vaccinated showed that it depends on the degree of contact with the source of infection. Of vaccinated persons who contracted brucellosis, 77.3% were workers tending sheep and goats during lambing and kidding, while another 12.7% were workers in the meat industry, and the remaining 10% had come into contact with their own privately owned animals, especially at lambing or kidding time.

According to figures given by Drankin & Simagina (1955), among vaccinated persons who contracted the disease, 33% did so during the first two months and 35% a long time after vaccination (nine months or more). On this basis the vaccination and re-vaccination periods have been arranged in such a way as to ensure that the vaccinated persons possess a more solid immunity by the beginning of the epidemic season. The clinical features of brucellosis among vaccinated persons are dependent on the interval between vaccination and the onset of illness. In persons who contract the disease less than two months after vaccination, or when more than a year has elapsed, the clinical course of brucellosis in no way differs from that of fresh brucellosis among the unvaccinated. In persons who contract the disease between two and nine months after immunization, a longer incubation period is noted, together with a gradual, non-acute initial stage, a shorter duration of the disease, and a milder course, often accompanied by a subfebrile temperature.

RELATIONSHIP OF BRUCELLOSIS IN SHEEP AND GOATS TO HUMAN CASES AND VACCINATIONS IN THE USSR, 1952-58

	1952	1953	1954	1955	1956	1957	1958
Incidence of brucellosis in goats and sheep (1952 = 100)	100	98.1	110.4	117.0	113.7	114.0	—
Number of persons vaccinated (in thousands)	2 421	4 741	8 305	10 851	21 371	25 031	3 177 677
Relative number of human cases (1952 = 100)	100	79.6	70.2	57.8	58.8	42.2	40.5

The published and reported figures for the last seven years from republics, oblasts, rayons, industrial enterprises and farms lead to the conclusion that the reduction in the incidence of brucellosis in the USSR (by 59.5% between 1952 and 1959) is due in the main to vaccination. The accompanying table shows that the reduction in the incidence of brucellosis is in correlation with the increase in the number of vaccinations, in spite of the fact that the epizootiological position remains unfavourable.

Revaccination is carried out a year after primary vaccination in order to re-establish solid immunity. When revaccination is done subcutaneously the vaccinated persons must first be classified according to their immunological reactions, and this makes prophylactic work extremely difficult. In view of this we studied cutaneous revaccination, using the live vaccine in 10 times as high a concentration as in subcutaneous injection (2000-2500 million). Trials of cutaneous revaccination (Vershilova, Feder & Polyakova, 1952; Drankin, 1957; Balandin, 1957) showed that it was highly effective and that it can be used without preliminary testing for immunological reactions; serious reactions in persons sensitized to the brucella antigen, as occurred in individual cases on subcutaneous revaccination, were not observed when the cutaneous method was used. Study of the material on cutaneous revaccination of 7125 persons (Vershilova & Golubeva, 1958) enabled us to recommend this method for extensive practical application. During the last two years it has completely replaced the subcutaneous method.

Experimental research by a number of authors has shown that it is possible to establish immunity by various methods of immunization with live brucellosis vaccine (subcutaneous, cutaneous, conjunctival, intranasal, and aerosol spraying). On the basis of this work trials of human vaccination with live vaccine by the cutaneous method have been carried

out in the USSR. The effectiveness of the method was demonstrated for the first time in a small-scale test by Zenkova (1956). In 1956-58 the cutaneous method of vaccination was studied in foci of brucellosis of sheep and goats and in the meat industry (Smirnov et al., 1958). A live vaccine made from *Br. abortus* strains 19 and variant 19-BA was used for cutaneous vaccination. From 2000 to 5000 million brucellae (as measured by the opacity standard) were found in one dose of vaccine of various batches. The usual method of cutaneous vaccination was employed. The research was concerned also with finding out whether it was possible to use the cutaneous vaccination method without preliminary checking for reactions of serum allergy. For this purpose groups of people with positive Wright and Burnet reactions at the time of vaccination were vaccinated.

Cutaneous vaccination against brucellosis of persons with negative reactions to brucellosis showed that the local reaction results in slight hyperaemia and infiltration (1.5 × 1.5 cm to 2.5 × 3 cm) in about 76% of those vaccinated, often with formation of vesicles on the site of the incisions. General reactions, according to subjective findings, in the form of headache and weakness occurred in 3%-7.3% of the vaccinated for 1-3 days. In people with positive Wright and Burnet reactions, local reaction to injection of the vaccine was recorded in 57%-85.6%. General reactions were noted in 32%-47.6%, including a rise in temperature (37.1°C-37.7°C) in 14.5%. Kasymova, Beklemishev & Uzbekova (1960) demonstrated that the intensity of local and general reaction to cutaneous vaccination depends on the degree of sensitization of the organism at the time of vaccination. The authors conclude that cutaneous vaccination with live brucellosis vaccine is harmless for people with positive Wright and Burnet reactions. Cutaneous vaccination is

contra-indicated in persons who have suffered from active brucellosis, since it causes strong reactions and may lead to exacerbation of the disease.

Cutaneous vaccination, including the question of its effectiveness, is still in the research stage, but the preliminary figures show that it is in no way inferior to the subcutaneous method in immunological effectiveness.

CONCLUSION

1. The data available in the USSR on human vaccination with live brucellosis vaccine made from *Br. abortus* strains 19-BA and 19 have shown its high epidemiological effectiveness against brucellosis contracted from sheep and goats. For that reason

the epidemic control services in the USSR are using antibrucellosis vaccination as one of the main prophylactic measures.

2. The next task in vaccine prophylaxis of brucellosis is to introduce cutaneous vaccination as a general method and to improve the method of producing dried cutaneous vaccine from *Br. abortus* strains 19-BA and 19.

3. In experimental research, among the most important tasks are the obtaining of new vaccine strains of high immunogenicity, the proving of the new vaccine strain *Br. abortus* 104-M, and continuation of research into the nature and mechanism of the immunity produced by live brucellosis vaccine.

RÉSUMÉ

La plupart des cas de brucellose humaine, en URSS, ont pour origine le contact avec les moutons et les chèvres infectés. Ces dernières années une action prophylactique intense a été entreprise, d'une part pour supprimer la maladie parmi ces animaux, et d'autre part pour protéger l'homme par la vaccination au moyen de vaccin vivant. Bien que la situation, chez les animaux, soit loin d'être favorable, la fréquence des nouveaux cas de brucellose humaine a baissé de près de 60% de 1952 à 1958. La comparaison, au cours de cinq années, de la fréquence des cas parmi les vaccinés et les non-vaccinés exposés à l'infection a donné une moyenne de 0,5% pour les premiers et de 12,3% pour les seconds. La vaccination a été étendue, dans des foyers de brucellose, à quelque 200 000 personnes, chez qui la fréquence de la maladie

fut 3,3 fois (et dans un cas 11,2 fois) moins forte que chez les non-vaccinés. La brucellose a même disparu chez certains groupes de travailleurs exposés, là où l'on a procédé à la vaccination du personnel nouvellement engagé. Le vaccin a été préparé à l'Institut Gamaleya, à partir de *Br. abortus* 19-BA et 19. L'immunité croisée entre souches de *Brucella*, c'est-à-dire l'existence d'une immunité non spécifique de type permet d'utiliser une souche bovine contre l'infection ovine et caprine. De nouvelles souches hautement immunogènes sont à l'étude. Le vaccin a été administré par voie sous-cutanée, mais il semble que la voie cutanée donne d'aussi bons résultats. Une seconde vaccination est effectuée un an après la première, afin de consolider l'immunité.

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