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COMPARISON OF LIVING VACCINES IN PRODUCING IMMUNITY AGAINST NATURAL BRUCELLA MELITENSIS INFECTION IN SHEEP AND GOATS IN IRAN

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Before a vaccination programme could begin in Iran against *Brucella melitensis* infection in sheep and goats, it was essential to determine which of several vaccines would give the best protection under local conditions. A smooth attenuated *Br. melitensis* strain, Rev 1, developed by Elberg, had been effective in goats. *Br. abortus,* Strain 19, which is widely used for vaccination of cattle has also been used in Russia in sheep.

Forty female goats and 93 fat-tailed Kurdi ewes, 14 to 16 months of age and non-pregnant, were purchased in the province of Azerbaijan. Five male Kurdi sheep and three male goats were also obtained from this area. An additional five female goats and six Merino ewes were obtained locally. All animals were negative to agglutintion, coplement fixation and allergic tests performed with brucella antigen.

On August 2, 1962, twelve goats and 30 sheep were vaccinated subcutaneously behind the right shoulder with 1 ml of Rev 1 vaccine (containing 2.2×10^{9} viable organisms). On the same day twelve goats and 25 sheep were vaccinated in a similar manner with 2.5 ml of Strain 19 vaccine (containing 40×10^{9} viable organisms). On September 1, five goats and six Merino sheep were vaccinated subcutaneously with 1 ml of *Br. neotomae*, Strain

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5K33 (containing 3×10^9 viable organisms), a culture obtained from Dr. H. S. Cameron, Davis, California, which had originally been isolated from a desert wood rat.

Sixteen goats and 38 sheep were not vaccinated.

All female animals were held together in an open pen until October when they were housed together in a brick stable. In October the males were put with the flock and natural breeding commenced. The males were left in the flock until the end of the experiment.

Five goats and 10 sheep from the non-vaccinated group were bred one month earlier than the remaining animals. When they were in their second month of pregnancy they were injected subcutaneously with 6×10^9 organisms of a strain of virulent *Br. melitensis* which had been isolated from a sheep which had aborted in Isfahan. Twelve of these animals aborted and two others had normal but infected parturitions. One was non-pregnant. These animals served as donors and thus provided a source of natural infection to the experimental animals. There were three abortions due to *Br. melitensis* in the nonvaccinated controls and two in the group vaccinated with Strain 19. In addition, there were three abortions of unknown cause.

At the time of parturition, or abortion, bacteriological examination was made of the placenta and the foetus. Kids and lambs were autopsied within 24 hours of birth and twenty tissues were removed from each and examined bacteriologically. Vaginal swab and milk samples were taken from the adult twice a week and were examined bacteriologically. The adults were autopsied about one month after parturition and 24 tissues were examined bacteriologically. All specimens were cultured on serun dextrose agar with antibiotics. One brucella colony from each source was tested to see if it was the challenge strain or a vaccine strain. Two colonies recovered from the hepatic lymph node and the liver of one sheep vaccinated with Rev 1 seven months previously were identified as Rev 1. All other isolated cultures were identical with the challenge strains.

A summary of the results of bacteriological examination of all materials is given in the table. It can be seen that in addition to the abortions in the control and Strain 19 groups, there were a number of infected parturitions. Brucellae were recovered from the placentae of a number of vaccinated animals which were negative on all subsequent bacteriological examinations. More kids and lambs were found to harbour *Br. melitensis* in their tissues than expected on the basis of subsequent infection data from their mothers. There is the possibility that some of the kids and lambs were not infected

in utero but within the first few hours after birth in the heavily infected environment. It was observed that infected kids and lambs born of vaccinated mothers frequently had infection limited to the regional lymph nodes and the numbers of burcellae recovered per tissue were less than those from the controls.

Following parturition, 13 control animals, three animals vaccinated with Strain 19 and one sheep vaccinated with Rev 1 became heavy excretors in milk and vaginal secretions. The spread of infection was such that two-thirds of the female controls and all of the non-vaccinated males were infected at autopsy. About half of the animals vaccinated with Strain 19 or with *Br. neotomae* were infected at autopsy. Only 5 of 42 animals vaccinated with Rev 1 were infected at autopsy and, in the case of three of these, less than 10 colonies were recovered per sheep.

Challenge by natural infection tests the ability of a vaccine to produce an immunity capable of withstanding repeated or continuous exposure. In this experiment there were excretors in the flock from the time of the first infected parturition in the donors (when the vaccinated animals were in their third month of pregnancy) until the last animals were autopsied five months later. At the time of heaviest infection there were 15 excreting animals in a stable of 175 sq. m. Under these conditions of heavy exposure it is unlikely that any vaccine could protect all animals. Rev 1 was, however, quite effective in preventing the establishment of brucella infection. Only one sheep vaccinated with Rev 1 developed a rising serological titre, followed by an infected parturition and excretion in the milk and vaginal swab for 25 days, but when autopsied five weeks after parturition only a few brucellae were recovered. Four other sheep vaccinated with Rev 1 had a few brucellae in their tissues at autopsy. In contrast, there were two abortions and three heavy excretors in the Strain 19 group and 50% were infected at autopsy. Not enough animals were vaccinated with *Br. neotomae* to make a valid comparison but it appeared to be about as effective as Strain 19. It was concluded that Rev 1 vaccine afforded better protection against natural exposure to *Br. melitensis* infection than Strain 19 vaccine.

Summary of Results of Bacteriological Examination

Challenge Strain of **Brucella melitensis** Isolated from:		Number positive/total number examined per group			
		Controls	Rev 1	Strain 19	**Brucella neotomae**
Aborted materials from	sheep	2/3	0/1	2/2	0
	goats	1/1	0/1	0	0
Placentae from	sheep	10/18	5/20	9/16	2/4
	goats	3/6	5/11	4/9	1/3
Tissues from lambs		8/20	7/27	12/21	3/6
Tissues from kids		6/8	9/11	4/11	1/5
Vaginal swab from	sheep	9/23	1/28	3/23	1/6
	goats	4/10	0/11	1/12	0/5
Heavy milk infection * from	sheep	10/23	1/27	2/23	0/6
	goats	3/9	0/11	1/12	0/5
Total milk infection ** from	sheep	15/23	6/27	5/23	1/6
	goats	7/9	2/11	6/12	2/5
Tissues from female	sheep	18/26	5/30	12/35	3/6
	goats	7/11	0/12	6/12	2/5
Tissues from male	sheep	5/5			
	goats	3/3			

* Heavy infection = animals from whom numerous colonies of *Br. melitensis* were repeatedly isolated from milk.

** Total infection = in addition to those with heavy infections, animals are included from whom a few colonies were isolated on one or two occasions from milk.