

Comparative tests with formolized and irradiated vaccines against leptospirosis*

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The effectiveness of gamma-irradiated antileptospirosis vaccines and of vaccines killed with formol was compared in guineapigs. Irradiated vaccines proved no more effective than formolized vaccines in preventing either death or persistent kidney infection. Anti-pomona vaccines gave little protection against serotype icterohaemorrhagiae, and there was no significant difference between irradiated and formolized preparations. Vaccine prepared from a virulent strain showed little advantage over vaccine prepared from weak strains.

Prophylactic killed vaccines have been widely employed against leptospiroses in both man and domestic animals. In the first instance the results obtained have generally been very satisfactory; in the second, users' findings have often differed. It has been stated that even if, in most cases, such a vaccine can protect animals from the disease, it cannot protect them from becoming infected, and therefore carriers of leptospires. We have investigated whether the use of vaccines inactivated by gamma radiation gave better protection and whether this protection extended to strains antigenically different from that employed for the preparation of the vaccine.

In recent years Hubbert & Miller (1965) and subsequently Stalheim (1967, 1968a, 1968b) have prepared an antileptospirosis vaccine by irradiating a suspension of virulent leptospires with appropriate doses of gamma rays. The leptospires are not killed by this treatment, but they become incapable of reproduction and lose their pathogenicity. These investigators found such vaccines to be effective, protecting animals both from the infection and from becoming carriers. In addition to small laboratory animals, Stalheim (1968a, 1968b) has also used this vaccine to protect a small number of cattle and swine. The dose of ionizing radiation selected by Stalheim was 100 000 rad.

We have repeated these investigations, comparing the effectiveness of an irradiated vaccine and of a formolized vaccine prepared by the usual methods.

We also attempted to determine whether vaccines prepared by the two techniques using (1) a virulent, recently isolated strain, and (2) an attenuated laboratory strain, would show a difference in activity. Finally, we investigated whether the two types of vaccine—attenuated live vaccine and formolized vaccine—could protect against leptospires of a different serotype.

MATERIALS AND METHODS

The following *Leptospira* strains were employed for these tests:

(1) strain Policlinico (serotype *icterohaemorrhagiae*), isolated from man by Babudieri in 1965 and maintained in alternate transfers in guineapigs and in Korthof-Babudieri's medium (Babudieri, 1961). This strain has maintained its virulence and is able to kill guineapigs.

(2) strain Dellatte (serotype *icterohaemorrhagiae*),³ isolated from man by Babudieri in 1940 and maintained since in culture. It has a low virulence and produces subclinical infection in the guineapig.

(3) strain New Jersey (serotype *pomona*), isolated from cattle in 1946 by Baker and Little. This is the strain most often used in the USA for the prepara-

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³ After these experiments were completed, Babudieri (*Ann. Ist. sup. Sanità*, 1972, 8, 159) reclassified strain Dellatte as belonging to serogroup *icterohaemorrhagiae*, serotype *copenhageni*.

tion of anti-*pomona* vaccine for cattle. It is maintained in culture and its virulence is very limited.

These strains were cultivated for 10 days at 30°C in Korthof-Babudieri's medium. The cultures were then centrifuged for one hour at 10 000 rev/min and the sediment was resuspended in the same culture medium, but without rabbit serum and vitamin B₁₂. The numbers of leptospire were counted in a Hawksley counting chamber, and the suspension was diluted to a concentration of 1×10^8 spirochaetes per ml.

Part of this suspension was treated with 0.05% formol. Another part was placed in a large test tube and irradiated with a cobalt bomb so as to receive 200 000 rad per ml. We chose this dose, instead of the 100 000 rad suggested by Stalheim (1967), because the lower dose did not appear sufficiently sure. One of the guineapigs inoculated by Hubbert & Miller (1965) with a vaccine irradiated with 50 000 rad developed a persistent kidney infection; we therefore considered that only a four-fold dose would be safe.

The absence of contamination in each vaccine batch was checked by means of transfers in the customary media and in Korthof-Babudieri's medium, and by darkfield examination. The irradiated leptospire were motile under the microscope, but were unable to multiply in Korthof-Babudieri's medium.

With each type of vaccine we inoculated a group of 20 guineapigs each weighing 175-280 g. The vaccine was inoculated in a single dose of 1 ml per guineapig. A group of 20 unvaccinated guineapigs was used as a control. On the 21st day all the guineapigs, including controls, received single intraperitoneal injections of 1 ml of a rich 10-day-old culture of the virulent strain Policlinico.

After vaccination, the guineapigs were kept under observation for 21 days in order to determine if they developed morbid symptoms due to the vaccine. Guineapigs that became infected were kept under observation for 23 more days. Post-mortem examinations were carried out on those that died; we sought the characteristic signs of *Leptospira* infection, and made haemocultures and cultures of liver and kidney tissue in Korthof-Babudieri's medium. All surviving guineapigs were sacrificed after the period of observation and subjected to post-mortem examination. Samples of kidney tissue were transferred to Korthof-Babudieri's medium, and the sera were examined for the presence of agglutinins.

In order to ascertain that the irradiated vaccine was harmless, 21 days after inoculation we sacrificed

4 guineapigs of each of the two groups that had received irradiated vaccine prepared with leptospire of the *icterohaemorrhagiae* serotype (strains Policlinico and Dellatte). With these guineapigs, too, we carried out autopsies and culture tests, and examined the serum for agglutinating antibodies.

The results were evaluated statistically by comparing the percentages obtained by the χ^2 test, using 2×2 frequency tables.

RESULTS

The irradiated vaccines used in the tests proved completely safe. They caused no evident pathological changes in guineapigs, and even the 8 animals sacrificed on the 21st day showed no anatomical or pathological alteration. The cultures prepared from kidney tissues proved negative. Agglutinins were not detected in the serum, even at a titre of 1:10. This is not surprising, as it is well known that the limited amount of antigen contained in one dose of killed *Leptospira* vaccine is usually unable to provoke an evident rise in antibody in guineapigs. The antigenic activity of live but irradiated leptospire, unable to multiply, corresponds to that of an equivalent amount of a killed antigen. Seven of the vaccinated guineapigs and 1 control animal died before challenge. Post-mortem examinations and cultures in no way indicated that their death could be attributed to inoculation with the vaccine. The sera of vaccinated guineapigs that survived until challenge agglutinated the two *icterohaemorrhagiae* strains at titres varying from 1:200 to 1:600.

The results obtained in the different tests are summarized in Table 1.

DISCUSSION

In evaluating the efficacy of an antileptospirosis vaccine, a distinction must first be made between protection against disease (and death) and protection against infection. In the first instance the lack of any symptom of disease does not exclude the possibility that the vaccinated person or animal might become a carrier and spreader of leptospire.

In man the carrier state is of no practical importance because it lasts for a very short time and usually does not constitute a source of infection for other people. In cattle and other domestic animals, vaccinated individuals that nevertheless become carriers spread leptospire in limited quantities and for a short period of time. In this paper, in the statistical

Table 1. Results of vaccination of guineapigs with gamma-irradiated and formolized vaccines and subsequent challenge with strain Policlinico

Vaccines	Vaccination				Challenge			
	Animals in group	Mortality in group	Animals sacrificed	Positive kidney cultures from sacrificed animals	Animals infected by injection	Mortality from leptospirosis	Surviving animals	Positive kidney cultures from survivors
Irradiated								
Policlinico	20	1	4	0	15	3	12 (80 %)	2
Dellatte	20	1	4	0	15	4	11 (73 %)	0
New Jersey	20	3	0	—	17	11	6 (35 %)	1
Formolized								
Policlinico	20	1	0	—	19	2	17 (89 %)	2
Dellatte	20	0	0	—	20	11	9 (45 %)	2
New Jersey	20	1	0	—	19	13	6 (31 %)	2
Unvaccinated controls	20	1	0	—	19	17	2 (11 %)	2

evaluation of the results, we took into consideration both partial protection (protection against illness and death) and complete protection (protection against illness and death and against the carrier state).

An analysis of the data in Table 1 indicates that there was no statistically significant difference between the protection afforded by *icterohaemorrhagiae* irradiated and formolized vaccines, when they were compared (partial protection, $\chi^2=0.4$; complete protection, $\chi^2=0.8$). The irradiated vaccine prepared with strain Policlinico was practically as effective as the corresponding formolized vaccine. The irradiated vaccine prepared with strain Dellatte gave protection to more animals than the corresponding formolized vaccine. However, the difference was not statistically significant (partial protection, $\chi^2=1.8$; complete protection, $\chi^2=3.6$).

Both in the groups that received irradiated vaccine, and in those treated with formolized vaccine, some of the surviving guineapigs became renal carriers of leptospirosis. The irradiated vaccines did not afford better protection against this condition than the formolized ones.

Statistical comparison showed that vaccine prepared with the virulent strain Policlinico may give greater protection than vaccine prepared with strains of low virulence, but only if we consider the criterion of partial protection ($\chi^2=5.3$), and not complete protection ($\chi^2=2.7$). Apart from the question of

virulence, it should be borne in mind that strain Policlinico was also used for the challenge, and the identity of the antigenic pattern could be a factor in the better result achieved by the Policlinico vaccines.

The two vaccines prepared with serotype *pomona*, although less effective than the *icterohaemorrhagiae* vaccines (partial protection, $\chi^2=12.3$; complete protection, $\chi^2=11.6$), clearly give protection against serotype *icterohaemorrhagiae*, at least as regards complete protection (partial protection, $\chi^2=2.3$; complete protection, $\chi^2=4$). In this case again, the irradiated vaccines did not prove more effective than the formolized vaccine.

This conclusion may be of some interest if related to the experiments by Kemenes (1964), who found that a formolized anti-*pomona* vaccine did not give any appreciable protection against infection by serotype *icterohaemorrhagiae*, while the protection was very reliable in animals that had overcome an infection by serotype *pomona*. This finding might have suggested that a live vaccine, although irradiated, could give a cross-immunity far superior to that given by a killed vaccine. However, this did not take place in our experiments, and the capacity to immunize of irradiated leptospires, which are incapable of reproduction, is a phenomenon quite different from the greater capacity of virulent and aggressive leptospires.

To conclude, it does not appear that gamma-irradiated antileptospirosis vaccine is more effective than the common formolized vaccines, at least in the guineapig, even for conferring protection against becoming a carrier.

As the irradiated vaccine cannot be kept for a long time, but must be employed at once after its preparation, it is evident that while irradiated vaccine can be used as a substitute for formolized vaccine, there are practical disadvantages.

RÉSUMÉ

ESSAIS COMPARATIFS DE VACCINS FORMOLÉS ET DE VACCINS IRRADIÉS CONTRE LA LEPTOSPIROSE

On a comparé chez le cobaye l'efficacité de vaccins anti-leptospirose vivants, irradiés par rayonnement gamma à la dose de 200 000 rad/ml, et de vaccins tués par le formol.

Les vaccins irradiés ne se sont pas montrés plus actifs que les vaccins formolés correspondants, même en ce qui concerne la prévention de l'infection rénale et de l'état de porteur de germes. Les vaccins anti-*pomona* n'ont

suscité qu'une faible protection contre l'infection par le sérotype *icterohaemorrhagiae*. Ici encore, les vaccins irradiés n'ont pas donné de meilleurs résultats que les vaccins formolés.

Les vaccins obtenus à partir de souches virulentes ne présentent apparemment que peu d'avantages par rapport aux vaccins préparés à l'aide de souches faiblement pathogènes.

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