ASIA 1 FOOT-AND-MOUTH DISEASE VACCINATION ON PIGS With FORMALINIZED SAPONIZED VACCINE

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Summary

Asia 1 Foot and Mouth disease virus is inactivated on pigs by a formalin percentage really lower than that required for cattle.

The same virus attenuated by formalin at the limit of the inactivating doses for pigs is a really good vaccine for this animal.

Swine vaccination with bovine FMD virus raises a lot of problems which the OIE FMD commission and FAO commission and FAO European Commission for the Control of FMD have frequently reported. We discussed this problem in our report (1) to the Singapore FAO meeting December 1963 on pig diseases.

Various solutions were tried to solve these difficulties: a more concentrated vaccine, double vaccination, use of swine virus. But the most valuable contribution seems to us to be Kubin's work (2) He produced good swine vaccine thanks to the concentration of a pig virus, thanks above all to the reduction of formalin percentage.

According to our experience (3) Asia 1 foot-and-mouth disease virus when inactivated for cattle requests less formalin than types O and A. Consequently our Asia 1 cattle vaccine is only attenuated with 0.025% formalin. With such a vaccine which protects 100% cattle at the laboratory control, we vaccinated 5 pigs. When controlling potency, made with 100 pigs ID 50 of homologous virus we protected only 1 pig. So we have tried to find out what is the formalin doses able to inactivate Asia 1 virus on pigs and then to use the doses just lower to produce vaccine.

I. Asia 1 FMD virus inactivation on pigs.

The virus was from bovine origin but passed 12 times on Frenkel culture. Inactivation was carried out from a culture extract, after heat action 26°C during

48 hours with various formalin quantities. Formalin percentages were 0.005% 0.010%, 0.025%, 0.050% respectively. Infectivity was searched by the foot pad route according to the method we described recently (4). Intradermal injections were carried out in 5 sites on each foot that is to say on 20 sites per pig. The virus infectivity titer was $10^{-6.5}$ on unweaned mice and $10^{-5.2}$ on pigs. See Table 1. Apparently Asia 1 virus was inactivated for pigs only with 0.01% formalin.

Table 1. Inactivation of Asia 1 for pigs by formalin.

% formalin	Number of pigs	Number of FMD lesions
0.005 %	4	50/80
0.010 %	4	0/80
0.025 %	5	0/100
0.050	2	0/20

II. Vaccination trial with reduced quantity of formalin

5 ml vaccined doses vaccine contained: 4.70 ml of 3000 mg. virulent epithelium extract, 25 mg saponin diluted in 0.25 ml phosphate buffer, 0.05 ml glycocol buffer. Formalin was added in the ratio of 0.005% just allowing attenuation but not inactivation. Vaccine was injected subcutaneously deeply behind the ears on 10 piglets. Only two animals showed abcesses with necrotic escharre, on the others there were no after-efects.

The immunity challenge was carried out with a bovine virus titer of which was $10^{-2.57}$/0.1 ml on pigs. We used about 50 DI^{50} for pigs. Table II note the reactions. With the exception of the two piglets with vaccined reaction (necrotic abcesses which can be avoided by a deep injection) the eight other were totally protected.

Discussion.

Some years ago Girard, Hirtz and Mackowiak (5) showed the importance of the infectivity threshold. Infectious unit, that is to say the minima infectious doses corresponding to the infectious titer is different to the virulent unit that is to say the virus particule. The morbid effect needs the action of a lot of

virulent particles. And, in front of the virus action, each organism has it own infection threshold. This is a part of the explanation of the various different titers of the same virus according to the species: cattle, pig, guinea pig. So, if the quality of a virus is known, the minimum virulent quantity acts alone. Then and contrary to the first assumption, an unpublished observation showed it was impossible to vaccinate an animal-a guinea pig in the present case with a repetition of the subinfectious doses. And anyway this subinfectious doses were still infectious for cattle.

In front of Kubiñ's and ours results, it is necessary to suppose that the idea of threshold reappeared but needed to be extended to the immunity But this immunity threshold seems to be more intricate. It depends not only on the concentration in immunigenic particles (6) but also on their degradation state. With formalin degradation some residual infectivity must still be maintained, and this last is defferent according to the various species. At this point, it is useful to evoke Schmidt's principle experiment. His vaccine (virus + alumine) protected guinea pigs well, but was still infectious for cattle. Our experiment with Asia 1 virus in cattle which requires less formalin that 0 and A virus for producing our effective vaccine, also acts in the same way.

Conclusion,

In order to be protective, the foot–and-mouth disease virus must be used beyond the infectivity threshold. What is more, it requires a minimum immunigenic concentration. But this concentration alone is not sufficient, and the virus must be degraded too much. And with formalin, degradation must signify attenuation and not inactivation. And the inactivating doses varies according to the species, the virus type and perhaps also-this fact needs to be demonstrated–from one source to another (Frenkel culture or monolayer tissue culture). (For references and table II see French text)