METHODS OF SURVEILLANCE FOR YELLOW FEVER - SEROLOGICAL STUDIES IN WILD AND SENTINEL ANIMALS

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Successive vaccination campaigns, both in Africa and the Americas, have made any interpretation of human serological surveys almost impossible as concerns yellow fever. At present it is generally the negative results which are most valuable since they enable the receptive population to be estimated and consequently the risk of an epidemic to be evaluated in accordance with other epidemiological data, the abundance of the vector in particular. However, the presence of the virus is necessary before there can be an epidemic and many questions arise in this connexion: where is the virus maintained in nature, how is it propagated, how can the extent of its spread be checked? Yellow fever is primarily a zoonosis: by the surveillance of wild vertebrates captured in given areas and the study of sentinel animals placed in selected sites not only can an inventory of wild populations liable to be affected by yellow fever virus be drawn up, but also an idea formed of the intensity of the circulation of the virus. This study has two aspects: attempts to isolate the virus, and serological study of exposed animals. It is the latter aspect which will be considered here.

Equipment and methods

The serological study of wild fauna and of sentinel animals poses certain special problems.

Wild vertebrates

As accurate a list as possible of the animal species in a given region must be established. Subsequently, systematic captures should be made in order to determine the density of the different animal populations.

The animals could be shot, but the effect of this type of "capture" on the presence of non-specific neutralizing substances in the serum of birds obtained in this way is well known (Scherer et al. 1964; Hardy et al. 1964). The ideal solution is to use non-wounding traps. By this means the animals can be captured, bled, and then marked and released. By taking successive samples from the same animal, such a study of the wild fauna in its natural habitat gives very useful additional information on the seasonal activity of the virus and on the duration of antibodies in the host.
Sentinel animals

Monkeys are still the sentinel animals of choice for yellow fever since by their use possible infections can be detected in the forest canopy. The mosquitoes liable to transmit the virus at this height are, in fact, not much attracted by laboratory mice, the other very frequently used sentinel animals. Chickens, rabbits, guinea-pigs and hamsters, which may have a selective attraction for certain vector arthropods, can also be employed.

Serum collection

Blood sampling techniques vary according to the method of capture and the zoological group. In the case of large animals killed or wounded by gun-shot the blood is taken by cardiac puncture with the minimum delay in order to avoid as far as possible the appearance of non-specific inhibiting substances. In the case of smaller animals captured alive several methods may be employed: cardiac puncture, carotid puncture, orbital sinus puncture, section of the axillary artery (rodents), section of the carotid artery (bats), or incision of the humeral vein (birds, bats).

The serum collected after coagulation is stored at -20°C. When very small amounts are sampled paper filter discs can be used. However the drawbacks of this technique are well-known: loss of sensitivity, deterioration of globulins (Bond et al. 1969; Cohen et al. 1969).

So as to avoid contamination of the sera as far as possible, which is essential to preserve their anticomplementary power, concentrated antibiotic solutions should be used during sampling.

Sero logical methods employed

It is desirable for all laboratories to employ standardized techniques for haemagglutination inhibition, complement fixation and neutralization.

As only limited amounts of serum are involved, it is necessary to use micro methods. Although well adapted to haemagglutination inhibition and complement fixation (Sever, 1962), they are not yet very well developed as concerns neutralization.

Haemagglutination inhibition (HI) remains the simplest screening method. Although there is not yet any universally employed system of serum treatment, acetone treatment gives the best results (Semenov et al. 1968).

Complement fixation (CF) does not present any technical difficulty provided uncontaminated sera are used, whence the necessity to collect the blood over antibiotics (or antiseptics). By using this reaction recent infections can be detected.

The neutralization test (NT) is still carried out for the most part on mice. It is urgently necessary to develop a tissue culture microtechnique which can be adopted by all laboratories.

Gel immunodiffusion can provide a rapid and cheap method for the detection of antibodies in wild vertebrates or sentinel animals (Papadopoulos et al. 1970).

Interpretation of the results

For the most part, interpretation must be based on the study of a single serum specimen per animal. In view of the antigenic relationships existing in group B, the serological interpretation should follow from a study of at least the three most commonly used tests (HI, CF, NT).
In the case of yellow fever by itself, interpretation will be easy and it will be possible to distinguish between recent attacks (positive CF) and older ones (negative CF).

If several antigens are involved, then interpretation becomes very difficult. The titre cannot be used as a guide, since heterologous reactions can exceed the homologous response in intensity, not only as regards the HI antibodies, but also very often the CF and even the NT antibodies.

Another factor involved in interpretation of animal serological tests is the duration of the antibody in the animal after an initial infection: this duration is sometimes very short even as concerns HI antibodies which normally appear at an early stage and persist for a long time.

Sentinel animals should be examined before being exposed in order to make sure that they have no immunity. Subsequently they should be checked at regular intervals. If this is done interpretation of the serological results is not too difficult.

Conclusions

The ideal in regard to yellow fever surveillance would be to detect the danger of a human epidemic at the moment when there is a recrudescence of the virus in wild fauna. Such fauna is varied. Monkeys, although they are still the best controls for circulation of the virus in nature are no longer the only animals involved. Outside the large forest areas, simian populations do not seem numerous or prolific enough to ensure continuous circulation of the virus. Moreover they acquire long-lasting immunity very readily. The results of serological surveys and experimental infections have led to the suspicion that other vertebrates are concerned in the maintenance of yellow fever virus during inter-epidemic periods, e.g. hedgehogs, carnivores, bats and birds.

Although we have no proof of their direct involvement in the forest cycle of yellow fever, studies of these vertebrates should continue since so far we do not yet know how yellow fever virus is maintained in nature in Africa.

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