STERILIZATION OF ARTHROPOD BLOODMEALS PRIOR TO BLOODMEAL IDENTIFICATION

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ABSTRACT. An investigation was undertaken to determine ways of sterilizing arthropod bloodmeals prepared on filter papers in order to destroy any potential pathogen without preventing host identification by the precipitin test. Chemical treatment with diethyl ether, acetone or chloroform or physical treatments with ultraviolet light or heat at 60° C proved to be satisfactory methods. The method of choice will depend upon the nature of the pathogen.

INTRODUCTION. Techniques for bloodmeal identification of arthropods are well established and have provided much useful information on the epidemiology of arthropod-borne diseases (Boreham 1972, 1975, and Tempelis 1975). At Imperial College, about 40,000 arthropod bloodmeals, received from all over the world, particularly the tropics and subtropics, are analyzed annually. The majority of these samples are smeared onto filter paper for ease of transport (Weitz 1956) but occasionally samples of whole insects are received in gelatin capsules (Snow and Boreham 1973).

The chances of introducing a pathogen with bloodmeal samples cannot be ignored. Such a risk is greatly increased when whole insects are transported from one country to another in dry ice or liquid nitrogen (Tempelis and Galindo 1970). In 2 recent instances it has been necessary to take special precautions to ensure that no viruses were present in arthropod bloodmeals before the samples were sent to England from Africa for processing. The first of these projects concerned bloodmeal identifications of *Ornithodorus moubata porcinus* collected from warthog burrows in East Africa, where it is known that the infection rate in the ticks with African swine fever virus is as high as 3.8% (Pierce 1974 and Plowright et al. 1969). The second piece of work where

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it was considered there was a marginal risk of importation of viruses and where special care was required concerned bloodmeal identification of *Culicoides* species in relation to the epidemiology of blue tongue virus.

Methods were therefore investigated by which bloodmeals of arthropods could be treated to inactivate pathogens prior to bloodmeal testing without destroying the serum proteins used in the precipitin reaction.

MATERIALS AND METHODS. *Anopheles stephensi* Liston was maintained in the laboratory at 30° C and 75–85% RH. The larvae were fed ground dog biscuits and the adults 10% glucose solution. All the mosquitoes used in the experiments were between 5 and 10 days old and had not received a previous bloodmeal. Mosquitoes were starved for 24 hr prior to feeding. *Stomoxys calcitrans* (Linnaeus) was maintained in the laboratory at 25° C and 70% RH. All flies used were receiving their first bloodmeal.

ARTIFICIAL FEEDING. Female *An. stephensi* were allowed to feed on the shaved side of a guinea pig anaesthetised with Nembutal while *S. calcitrans* of either sex were fed from a pipette on pig's blood which had been collected from a local slaughter house.

After the insects had fed to repletion they were maintained at 30° C for mosquitoes and 25° C for *Stomoxys* for various periods of time prior to killing and smearing the abdominal contents on Whatman No. 1 filter paper. The filter papers were stored at room temperature in a desiccator.

TREATMENT OF BLOODMEALS. Filter papers containing bloodmeals were subjected to 8 different treatments which are known to be deleterious to a variety of pathogens.

1. Immersion in diethyl ether for 1 hr.
2. Immersion in a 1% alcoholic solution of deoxycholic acid for 1 hr.
3. Heating at 60° C for 10 min.
4. Exposed to ultra-violet light (Wavelength 2537Å) for 15 min at a distance of 10 cm.
5. Immersion in β-propriolactone for 1 hr.
6. Immersion in chloroform for 1 hr.
7. Immersion in formaldehyde solution for 1 hr.
8. Immersion in acetone for 1 hr.

After treatment the filter papers were allowed to dry at room temperature and the bloodmeals cut out and eluted in saline and tested by the capillary ring test to determine whether bloodmeal identification could still be effected (Boreham 1975). Specific antisera to pig and guinea pig were prepared by lymph node injections of sera into rabbits (Boreham and Gill 1973). These antisera had titres greater than 1 in 100,000 and did not cross react with unrelated sera at a 1 in 10 dilution.

RESULTS AND DISCUSSION. Results are presented in Tables 1 and 2. Under laboratory conditions bloodmeals of *An. ste-*

Table 1. Effect of various treatments on bloodmeal identification of *Anopheles stephensi* Liston bloodmeals smeared on filter paper. Figures represent the number of blood meals out of 10 positive by precipitin test.

	Digestion Time (hr)									
Treatment	1	2	4	8	16	20	24	36	48	60
Control	10	10	10	10	10	10	5	4	3	0
Diethyl ether	10	10	10	10	10	8	6	4	2	0
Deoxycholic acid	10	10	10	10	9	8	1	0	0	0
Heat	10	10	10	10	10	10	6	5	2	0
UV light	10	10	10	10	10	10	4	3	0	0
β-propriolactone	10	7	6	3	5	3	1	2	0	0
Chloroform	10	10	10	10	10	7	1	4	2	0
Formaldehyde	0	0	0	0	0	0	0	0	0	0
Acetone	10	10	10	10	10	9	5	3	1	0

Table 2. Effect of various treatments on bloodmeal identification of *Stomoxys calcitrans* (Linnaeus) smeared on filter paper. Figures represent the number of blood meals out of 10 positive by precipitin test.

	Digestion Time (hr)							
Treatment	2	4	8	12	16	24	30	36
Control	10	10	10	10	10	7	0	0
Diethyl ether	10	10	10	10	10	10	0	0
Deoxycholic acid	10	10	10	10	8	3	0	0
Heat	10	10	10	10	9	9	0	0
UV light	10	10	10	10	10	8	0	0
β-propriolactone	10	3	3	0	0	2	0	0
Formaldehyde	0	0	0	0	0	0	0	0

phensi could be detected up to at least 20 hr after feeding and for *S. calcitrans* 16 hr after feeding. Subsequently only a proportion of the meals could be identified due to digestion. Treatment of the bloodmeals with diethyl ether, acetone or chloroform for 1 hr or subjecting the meal to sterilizing UV light or heat at 60° C for 10 min had no deleterious effect on bloodmeal identifications. Treatment with deoxycholic acid had a slight deleterious effect whereas a marked decrease in the number of bloodmeals identified occurred when β-propriolactone was used. Treating bloodmeals with formaldehyde completely prevented bloodmeal identification.

There are thus a number of different methods that may be used to treat smears on filter paper to destroy any pathogen present. The majority of pathogens are very susceptible to desiccation and the chances of importing them with bloodmeals are very slight, especially since most bloodmeals are stored for several days before transport. Protozoa such as species of *Leishmania, Plasmodium* and *Trypanosoma* are destroyed by drying but some spore forming bacteria such as anthrax are not. Some viruses such as swine vesicular disease are also very resistant to drying. Blue tongue virus is particularly resistant to desiccation and to chemicals and like the virus of epizootic haemorrhagic disease of deer is not destroyed by ether (Boorman, personal communication). However it is known that cell sheets on coverslips containing blue tongue virus are sterilized by treatment with acetone at —20° C for 1 to 2 mins (Pini et al. 1968). The low temperature was to preserve the cells, and the inactivation of the virus was not dependent upon this.

African swine fever virus is known to survive for years when dried at room temperature or frozen on skin or muscle but it is inactivated by heating at 55° C for 30 min or 10 min at 60° C. Although it is destroyed by ether it is resistant to many disinfectants (Andrewes and Pereira 1972).

The range of procedures described here to treat blood meals should be suitable to ensure inactivation of most pathogens. We have successfully used treatment with ether and acetone to sterilize bloodmeals without affecting the success of bloodmeal identifications. The method of choice for sterilizing bloodmeals will depend upon the nature of the pathogen concerned, and the availability of the various chemicals and sterilization procedures. The methods described here are cheap and easy to carry out under field conditions.

The chances of a pathogen actually infecting a host, even if present, will be very small in many cases. Many pathogens will be host specific and require a certain number of infective organisms to produce disease. In other cases the risk of infection through air-borne particles or handling infected materials will be greater and in such cases, in addition to sterilization procedures, it would be wise to handle materials in a safety cabinet and sterilize all glassware and reagents at the end of the test. All discarded bloodmeals, filter papers, etc. should also be autoclaved.

Such additional procedures would be especially indicated when nitrogen-preserved material is being processed.

All the experiments have been carried out on bloodmeals smeared onto filter paper and it should be noted that these methods may not be applicable to whole insects, where it would be difficult to ensure that the chemical or physical treatment reaches a virus in the middle of the bloodmeal. Under such circumstances a virus may be protected from the chemicals by the surrounding proteins.

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