

Sholdt, L. L., R. H. Grothaus, H. K. Gouck and C. E. Schreck. 1975. Field studies using repellent-treated wide-mesh net jackets against *Glossina morsitans* in Ethiopia. East African Med. J. 52(5):277-283.

Travis, B. V. and F. A. Morton. 1946. Treatment of clothing for protection against mosquitoes. N. J. Mosq. Extermin. Assoc. Proc. 33:65-69.

## 1263 9

### SALINE AS A COLLECTING MEDIUM FOR *CULICOIDES* (DIPTERA, CERATOPOGONIDAE) IN BLOOD FEEDING AND OTHER STUDIES

A. R. WALKER AND P. F. L. BOREHAM

Veterinary Research Laboratory, P.O. Kabete, Kenya; and Department of Zoology and Applied Entomology, Imperial College of Science and Technology, London, SW7 2AZ, England.

**ABSTRACT.** A technique is described for collecting *Culicoides* in saline which facilitates the sorting of material and is suitable for blood meal studies, arbovirus isolations and most entomologi-

cal investigations. Precipitin tests can be satisfactorily carried out on specimens maintained at room temperature in saline for periods not exceeding 48 hr.

**INTRODUCTION.** Saline has been used routinely as a collecting medium for *Culicoides* which have been studied in Kenya for the last 5 years in relation to the epidemiologies of bluetongue disease of sheep and ephemeral fever of cattle. It has proved suitable for collecting these insects in any studies where fresh, but dead, material is required, such as population estimates, blood meal identifications, age-grading, and virus isolation.

**MATERIALS AND METHODS.** *Culicoides* were caught in "Monks Wood" light traps (Service 1970) and CDC light traps (Sudia and Chamberlain 1962), with 500ml wide-necked polythene bottles fitted at the base of the gauze funnels. About 300ml of saline was used in each bottle; the unbuffered saline was made up at a concentration of 7.5g/l, with "Savlon" antiseptic (cetrimide active ingredient) at a 1:200 dilution to act as a wetting agent and as a cleansing and bactericidal agent. No screens were fitted to the traps; the occasional large catches of moths and beetles rarely swamped the bottles completely and they were normally immersed in the saline rapidly and did not harm the *Culicoides*. Simuliids were also caught

in good condition but mosquitoes tended to be damaged by abrasion of their wing scales and stress on their longer appendages.

On return to the laboratory the collections were cleaned and sorted by flooding them several times with tap water in a four liter jar. The light beetles, moths and scales were decanted to leave the denser *Culicoides* and other small insects in a fairly clear liquid. All age-grades and species of *Culicoides* sank rapidly in tap water. The collections were transferred to saline in white trays marked with numbered grids and the size of *Culicoides* catch could easily be estimated by spreading them out evenly and counting the totals in randomly chosen grid sub-samples. If necessary, specimens were transferred to alcohol for subsequent taxonomic examination.

Under a low power stereomicroscope with incident lighting against a white background the blood engorged individuals were very conspicuous and were picked out from the saline with a small spatula, washed in water and then dried whole for dispatch to one of us (PFLB) for precipitin testing to determine their

hosts. Results of 682 such tests have already been reported (Walker and Boreham 1976). Most meals were satisfactorily identified but 101 gave negative results and these were probably due to either old or partly digested blood or very small blood meals. However, some negatives may have been due to the deterioration of the blood while the specimens were stored in saline. The average length of time specimens were kept in saline before sorting and drying was 12 hr, from midnight of the collecting night to noon of the next day when sorting was complete. However some specimens caught early in the evening could be 18 hr old and even 24 hr old if sorting was delayed. Most of this time the saline would be at ambient temperatures of 20–25°C.

The maximum time that engorged specimens could be maintained in saline without affecting the precipitin test was determined by making a normal collection of *C. schultzei* (End.) at a pen with cattle, sheep, and goats and then storing the engorged specimens in the saline collecting medium for various periods of time at ambient temperatures (min. 18° max. 24°). Bloodmeals were analyzed to determine whether the host could be identified or whether deterioration had occurred, using standard techniques (Weitz 1956, Boreham 1972).

**RESULTS AND DISCUSSION.** The results are shown in table 1. They indicate that immersion of blood-fed specimens in saline for up to 48 hr had very little effect on

blood meal identification but after that time there is a marked drop in the number of specimens identified. There is also evidence that, after 36 hr and 48 hr in saline, a proportion of blood meals cannot be identified to host species but only to the family Bovidae thus implying that some elution of host protein had occurred in some specimens. It is obviously better to keep the samples in saline for as short a period as possible, but collection trips to distant sites are possible without special equipment to preserve the catches.

After 84 hr there were no specimens left in which the original blood meal was intact; the disappearance of the blood meal varied greatly, presumably depending on how long it took the gut wall to decay and rupture but once this had happened the blood was eluted rapidly.

Specimens for age-grading were very clear in the saline and samples were sorted by the criterion of their abdominal pigmentation (Dyce 1969); engorged specimens that were pigmented (i.e. parous) were also fairly obvious. Specimens maintained for an average of 12 hr in saline were suitable for dissection for examination of follicle relics but the fresher the specimen then the easier was the dissection.

Material collected in saline was also routinely used for arbovirus isolations by F. G. Davies at the Veterinary Research Laboratory, Kabete. The survival of virus in specimens stored in saline could not be tested directly for lack of *Culicoides* colonies but it has been shown (Luedke

Table 1. Effect on blood meal identification of storage of specimens of *C. schultzei* in saline.

Average time in saline (h)	No. tested	No. positive	Identifications
12	20	19 (95%)	18 cow, 1 sheep/goat <sup>1</sup>
24	20	20 (100%)	19 cow, 1 unidentified bovid <sup>2</sup>
36	20	18 (90%)	16 cow, 2 unidentified bovid
48	20	18 (90%)	10 cow, 2 sheep/goat, 6 unidentified bovid
60	20	6 (30%)	4 cow, 2 unidentified bovid
72	20	1 (5%)	1 unidentified bovid
84	20	0	

<sup>1</sup> Sheep or goat; these two hosts cannot be readily separated.

<sup>2</sup> A weak feed derived from the family Bovidae. It could be derived from cows, sheep or goats.

and Jones 1972) that bluetongue virus survives for months in *C. variipennis* (Coquillett) in buffered saline at 4° C in the presence of antibiotics. This method has been successful in Kenya for virus isolation and to date ephemeral fever and Palyam group viruses have been isolated using collections made in saline, (Davies and Walker 1974; Davies in preparation). For prolonged sampling at sites distant from the laboratory the samples in saline were cooled, or collected live then aspirated from the cages and stored in saline in tubes over ice. Sorting in the laboratory was followed by washing five times in phosphate buffered saline and removal of blood-engorged specimens but the sorting by means of a spatula was slow and a mechanised device (Dyce et al. 1972) is really needed to sort out large pools from saline.

**ACKNOWLEDGMENTS.** We are grateful to the Ministry of Overseas Development, U.K., financial assistance with the precipitin testing. This paper is published with the permission of the Director of Veterinary Services, Kenya.

#### Literature Cited

- Boreham, P. F. L. 1972. Serological identification of arthropod bloodmeals and its application. *Pans.* 18:205-209.
- Davies, F. G. and Walker, A. R. 1974. The isolation of ephemeral fever virus from cattle and *Culicoides* midges in Kenya. *Vet. Rec.* 95:63-64.
- Dyce, A. L. 1969. The recognition of nulliparous and parous *Culicoides* without dissection. *J. Austral. Entomol. Soc.* 8:11-15.
- Dyce, A. L., Standfast, H. A. and Kay, B. H. 1972. Collection and preparation of biting midges (fam. Ceratopogonidae) and other small Diptera for virus isolation. *J. Austral. Entomol. Soc.* 11(2):91-96.
- Luedke, A. J. and Jones, R. H. 1972. Storage of bluetongue virus infected *Culicoides variipennis*. *Amer. J. Vet. Res.* 33:1875-1878.
- Service, M. W. 1970. A battery operated light trap for sampling mosquito populations. *WHO Bull.* 43:635-641.
- Sudia, W. D. and Chamberlain, R. W. 1962. Battery operated light trap, an improved model. *Mosquito News.* 22:126-129.
- Walker, A. R. and Boreham, P. F. L. 1976. Blood feeding of *Culicoides* in Kenya in relation to the epidemiology of bluetongue and ephemeral fever. *Bull. Entomol. Res.* 66: (in press).
- Weitz, B. 1956. Identification of blood meals of blood sucking arthropods. *WHO Bull.* 15:473-490.

### THE SOUTH COOK COUNTY MOSQUITO ABATEMENT DISTRICT

155th Street and Dixie Highway

P.O. Box 30, Harvey, Illinois 60426

#### Board of Trustees

GEORGE J. CULLEN—President  
 LAWRENCE P. GULOTTA—Secretary  
 CYNTHIA L. HUMES—Treasurer  
 CLARENCE BOBBE—Vice President  
 FRED MASSAT—Vice President

**The District has served South Cook County Illinois since 1954.**