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AN AGAR GEL DIFFUSION METHOD FOR THE IDENTIFICATION OF MOSQUITO BLOOD-MEALS[1]

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Serological techniques based on the precipitin reaction have been widely used since Nuttall established the importance of the antigen-antibody system for the identification of unknown bloods. Arthropod blood-meals are routinely identified by subjecting a saline extract of the gut contents of an engorged insect to multiple tests with suitable antisera. When used for the identification of mosquito blood-meals, the system is generally conducted either in small diameter test tubes as reviewed by Weitz (1956) or in capillary tubes according to the method described by Tempelis and Lofy (1963). A precipitate formed at the interface between antigen and antiserum constitutes a positive reaction.

The agar gel diffusion technique described by Ouchterlony (1948) involves the diffusion of antigen and antibody through agar gel so that precipitate reactions leave a distinct precipitate band in the gel which is easily read even many days after the test has been conducted. The method is routinely used in systematic serology but until recently has not been applied to the identification of arthropod blood-meals. Chamberlain and Sudia (1967) describe an agar gel method applicable to the identification of mosquito blood-meals and cite some of the advantages of the technique.

The method described herein differs in many respects from techniques used by other workers. This system has been used to identify more than 10,000 mosquito blood-meals in our laboratory and has proven most satisfactory for this purpose.

MATERIALS AND METHODS. The agar preparation used in these tests consists of

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers—The State University of New Jersey, New Brunswick, N. J. 08903.

a 1.5 percent solution of Noble-Special Agar [2] mixed in physiological saline. Gel strength is sacrificed using this brand of agar but the clarity obtained eliminates the filtration steps often necessary with other types. The mixture is prepared over a low heat and should be continually stirred to prevent scorching and subsequent discoloration. Prior to pouring the plates but after the mixture has been removed from the heat Merthiolate (1:10,000) is added to prevent bacterial growth.

Plates are prepared by transferring 8.5 ml of the agar solution to individual 100 x 10 mm disposable petri dishes. The plates may be prepared well in advance and it is most convenient to pour enough units at one time to cover several weeks' testing. When cool, the plates are covered and refrigerated until used.

Prior to testing, wells to hold antigen and antisera are cut in each plate using a five hole Feinburg agar gel cutter.[3] The resultant pattern includes four wells 7.0 mm. in diameter arranged in a circular pattern around a 9.5 mm diameter center well. Approximately 7.0 mm separates the outside wells from the center well (Fig. 1). Two such patterns can be cut on each plate. Should conservation of either antigen or antisera be critical, similar patterns using smaller wells can be designed.

The mosquito blood-meal is extracted in 0.5 ml saline. A portion of this unknown antigen is placed in each of the two center wells on a plate. Each mosquito blood-meal is thereby simultaneously tested for eight different hosts by placing a different antiserum in each of the eight surrounding wells. If polyvalent screening antisera are used, each blood-meal can be screened for most hosts in a single test.

Plates are covered and allowed to stand overnight at room temperature. Diffusion of the antigen and antisera takes place through the agar so that the blood-meal extract makes contact with each of the antisera mid way between wells. Homologous reactions leave a white precipitate band in the agar gel (Fig. 1). Tests are usually read and recorded the following day but can be left for nearly one week if necessary. Special lights are available for reading the plates but reactions are easily visible when held up to a dark background. A 3- to 4-foot black square painted on the laboratory ceiling is most satisfactory.

Positive tests for polyvalent antisera are rerun with specific antisera to each animal in the positive screening group. This can be accomplished either through agar gel or by other methods such as the capillary tube technique. Negative tests can be rerun using different antisera.

DISCUSSION. Precipitin tests conducted through agar gel allow simultaneous reading of all tests and thereby provide a ready basis for comparing results. Since tests for all feedings are set up before any results are known, all hosts are assured equal consideration. This type of system is invaluable when the detection of multiple feedings is pertinent to the study. Questionable patterns can be set aside for later observation or even photographed for future study. The fact that tests do not need to be read at specific time intervals and can be left untended for many days is an important consideration for researchers who have field obligations which keep them from a standard laboratory schedule.

Blood-meal identification by agar gel diffusion is not entirely without drawbacks. Chamberlain and Sudia (1967) point out that agar gel diffusion may be 2-fold less sensitive than the capillary tube method but conclude that other advantages such as the use of unclear antisera and ease of reading tests compensate for this loss. The decreased sensitivity of the system does not imply that cross reactions interfere with results. Titration of an antiserum through agar gel rarely exceeds 1:4000 (1:10,000+ in the capillary tube) but heterophilic reactions likewise demonstrate a substantial drop in titer. Mos-

[2] Difco Laboratories, Detroit, Michigan.
[3] #1802, Consolidated Laboratories Inc., Chicago Heights, Illinois.

quitoes extracted in 0.5 ml. saline rather than the standard 1.0 ml. fall well within the limits of this procedure.

Clarity of the antisera is a point worthy of consideration. An antiserum which is partially hemolized or cloudy may be perfectly suitable for blood-meal identification but will visually interfere with the zone of reaction in the capillary tube or ring test. Commercially prepared lyophilized antisera often remain cloudy after mixing. Diffusion through agar gel filters these antisera.

Clarity of the blood-meal is actually more important. Blood-meals expressed on filter paper often leave bits of paper in the extracted sample and must be centrifuged before testing. Whole abdomens extracted in saline are frequently cloudy particularly if ovarian development has begun or if very small amounts of blood require mechanical extraction. Diffusion through agar gel eliminates centrifugation and allows the testing of all blood-meals including those of partially engorged females. Selecting only fully engorged specimens, as is often done for precipitin tests, could result in an inaccurate report of the feeding habits of a species in nature. A species known to feed readily on a particular group of animals may not always engorge fully on other hosts. Discarding partially engorged specimens in the laboratory could result in amplifying a preferred feeding pattern by mechanically eliminating atypical hosts.

Numerous samples which appear weak often do give positive reactions. Samples

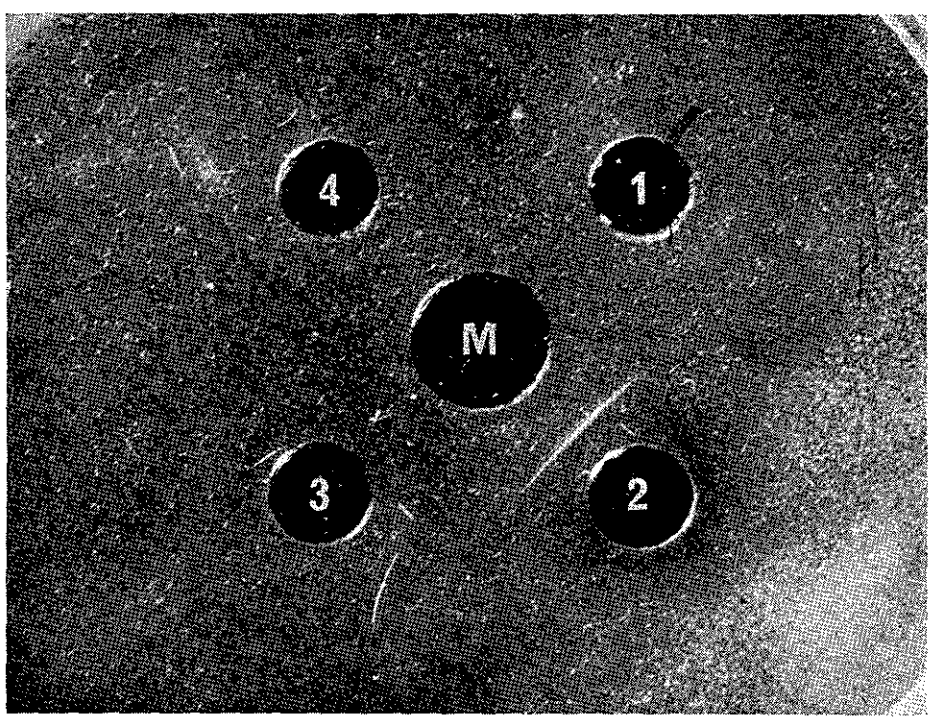

FIG. 1.—Agar gel diffusion system in a single test pattern showing positive precipitate reaction between wells "M" and "2".
Legend: M—Mosquito extract representing unknown antigen.
1 through 4—Wells containing representative antisera.

which are obviously weak or partially digested can be pre-recorded as such prior to testing so that they can be distinguished from full blood-meals which do not react. A separate category for weak samples not expected to react can thus be constructed.

SUMMARY AND CONCLUSIONS. Agar gel diffusion is a suitable method for the identification of mosquito blood-meals. The system has several advantages over other techniques particularly for screening large numbers of specimens. In many circumstances, a combination of agar gel for broad screening and capillary tube identification of specific hosts is advisable.

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EFFECT OF FELLING MANGROVES ON EMERGENCE OF *CULICOIDES* SPP. IN JAMAICA

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INTRODUCTION. In Jamaica, three species of biting sand flies (Diptera:Ceratopogonidae) are commonly associated with tidal mangrove swamp. *Culicoides furens* Poey and *C. barbosai* Wirth and Blanton are the main nuisance species, while a third, *C. insignis* Lutz is present in low numbers and only very rarely bites man.

An investigation into the breeding distribution of these species in which 16 emergence traps were placed in a transect across a 70-yard width of tidal swamp for 46 days showed that *furens* was associated with the drier white or black mangrove belts. *C. barbosai* tended to be confined to the seaward red mangrove belt and *insignis* was generally distributed across the swamp (Davies 1967).

When the original data from the above survey were grouped according to the shadiness of each trap site the results indicated that there was an association between shade and the numbers of each species caught, as shown in Table 1.

In the table, "shade" indicates traps that were shaded for more than three-quarters of the day; "half shade," for between a quarter and three-quarters of the day; and "open," for less than a quarter of the day. Because the distribution of *furens* was limited to the drier part of the swamp it was taken in only 12 of the 16 traps. The data in the table have been adjusted to allow for this.

The results show that while all species preferred some degree of shade there were great differences in the degree of preference. Compared with the "open" sites, *barbosai* was 22.8 times more abundant in the shade, while *furens* was less sensitive, with only 2.87 times as many. On the other hand *insignis* was more abundant in the "half shaded" habitat.

Whether this preference was determined by the presence or absence of shade alone or whether another factor was involved was not known. There was an obviously greater humus content in the

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