Antibodies to Antigens of *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) in the Sera of Infested Sheep: A Serological Technique for the Detection of Screwworm Myiasis[1]

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ABSTRACT *Antibodies to screwworm, Cochliomyia hominivorax* (Coquerel), antigens are found in serum of previously infested host sheep and for at least one month after infestation. ELISA provides a serological technique for detecting previous exposure to screwworm in noninfested hosts. The technique is adaptable to a field situation and has potential as a method for screening noninfested hosts for indirectly estimating infestation rates.

KEY WORDS Screwworm, ELISA, antibodies, myiasis, *Cochliomyia hominivorax*.

The screwworm, *Cochlimoyia hominivorax* (Coquerel), is an economic pest of livestock in the New World tropics. However, it is also a parasite of native and feral mammals. Wild hosts pose a serious problem to eradication program managers because they are not amenable to inspection, treatment and quarantine as are livestock. The wild host problem could become increasingly important as the eradication program moves from primarily agricultural areas into primarily forested areas. The degree to which native or feral animals support screwworm populations is largely unknown. In temperate North Carolina before eradication, Lindquist (1937) reported a 4% screwworm infestation rate in wild animals, mainly rabbits, deer and opossums. The rate of infestation is equivalent to what occurs in domestic animals in tropical southern Mexico. Thomas (1987) reported screwworm infestation rates in cattle, dogs and pigs at 1.7%, 4.1% and 5.3%, respectively. Although it would be impractical to survey large numbers of wild animals to directly determine infestation rates, a serological technique which would detect a history of previous exposure to screwworms would greatly reduce the sample size necessary to obtain estimates of infestation rates.

Serological testing depends on the presence of screwworm specific antibodies in the host blood and its persistence at detectable levels for a considerable length of time after an infestation. Borgstrom (1938) and Laake and Smith (1939) demonstrated that reinfested guinea pigs tolerated heavier infestations by screwworms than did naive quinea pigs. Those studies, however, lacked serological evidence for an immune response to an infestation. Larvae of the sheep blowfly, *Lucilia cuprina* (Wiedemann), which like the screwworm causes a subcutaneous

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myiasis, have been shown to induce an immune response in the host (O'Donnell et al. 1980, Sandeman et al. 1987), i.e., the production of serum antibodies specific to parasite antigens.

In the present study we analyzed the serum of screwworm infested and previously infested sheep for antibodies to screwworm antigens. We suggest that the serological test for anti-screwworm serum antibodies could be used in the field to survey the incidence of screwworm myiasis.

Methods and Materials

Animals. Eleven adult sheep (Barbados x Merino hybrids), with a recent history of 1-4 controlled episodes of screwworm infestation, were chosen for this study. Sheep were ear-tagged for individual identification.

Infestation and Serum Collection. Six of these sheep were placed in stanchions and artifically infested by placing approx. 20 1st-instar screwworm larvae into a small (2 cm diam) superficial wound on the haunch. Prior to transfer to the wound, these 1st-instar larvae were maintained on a bovine-blood gel media. Third-instar larvae were removed from the wound on the fourth day after infestation, the wound treated, and the sheep released from the stanchion. All infestations in all sheep were managed in this manner.

Blood was collected from the sheep by venipuncture, allowed to clot and serum separated by centrifugation. Serum was stored at -20°C. Blood was collected from the six infested sheep on the second day of the infestation. To test for residual antibody, blood was collected from the five uninfested sheep ca. 1 month after completion of their most recent infestation. A negative serum sample was obtained from a multi-sheep pool of serum collected at a slaughter facility in San Angelo, TX.

Antigen Preparation. Third-instar crawl-off larvae, laboratory reared on a bovine-blood gel media (Taylor and Magan 1987), were used for antigen preparation. Larvae were homogenized with a Ten-Broeck tissue grinder in 10 mM Tris-HCl, pH 7.5. Homogenate was centrifuged at 48,000 x g for 45 min at 4°C. The supernatant fluid was collected and filter sterilized by passage through a 0.45 μm membrane (acrodisc, Gelman Sciences). Protein concentration was determined by a modified Lowry technique (Bradshaw 1966). Antigen preparations from screwworm larvae (SW1) were stored at -70°C.

Enzyme-Linked Immunosorbent Assay (ELISA). The dry plate ELISA (field test) was performed as described. SW1 antigens diluted in distilled water (100 ug/ml) were added (50 ul, 5 ug/well) to each well of a microtiter plate (Linbro ISBN-96). The plate was air dried over night at 30°C. The plate was rinsed once with ELISA wash (0.15 M NaCl, 0.5% Tween 80) and blotted dry just prior to use. Sheep serum to be tested was diluted 1:20 in serum diluent (0.01 M sodium phosphate buffer, pH 7.2 containing 0.15 M NaCl, 2% non-fat dry milk, 1% Tween 80, and 0.01% antifoam A). Diluted sheep serum (100 ul) was added to each well and incubated for 10 min with shaking (Titertek plate shaker, Flow Laboratories, Inc., McLean, VA) at room temperature. The plate was then rinsed three times with ELISA wash and blotted dry. Peroxidase conjugated rabbit anti-sheep IgG (Cooper Biomedical, West Chester, PA, H+L chain specific) diluted (1:1000) in serum diluent was added (100ul) to each test well and incubated for 5 min with shaking at room temperature. The plate was then rinsed three times with ELISA

wash and blotted dry. Substrate (100ul) (0.2 M ABTS [2,2'-azino-*bis* (3-ethyl) benthiazoline sulfonic acid], 2 mM H_2O_2, 0.05 M citrate (pH 4), was added to each well and the plate incubated 10 min with shaking at room temperature. Absorbances of the developed color reactions were determined at 414 nm with a Titertek Multiscan plate reader (Flow Laboratories, Inc., McLean, VA).

Results

Block Titration for Dry Plate, Field ELISA. A checkerboard "block" titration was performed by diluting antigen by columns and test serum by rows of the microtiter plate (Voller et al. 1989). A clear distinction between positive serum (anti-SWl) and negative serum was achieved at 5 µg SWl per well and a serum dilution of 1:20 (Fig. 1) as mean absorbance was greater than 2X the negative control.

Storage of SWl Dry Plate. SWl antigen bound to microtiter plates remained antigenically stable and field ELISA absorbance readings for high, moderate, or low concentrations of anti-SWl antibody only varied slightly when the sensitized microtiter plate was stored at room temperature for 4 weeks (Fig. 2).

Evaluation of Infested Sheep Serum with Dry Plate ELISA. Anti-SWl antibody was found in the serum of all sheep that had been exposed to srewworm larvae after at least one infestation episode (Table 1). Residual anti-SWl antibody was detectable in sheep serum for at least 38 days after the initial infestation.

Table 1. Anti-SWl antibodies in serum from sheep previously infested with screwworms. Group 1 sheep had a current infestation. Group 2 sheep were sampled ca. 1 month after their most recent infestation.

	Animal	Previous infestations	Days Post-infestation†	Mean Absorbance*	SD
Group 1 infested	74	1	2	0.334	.008
	55	3	2	0.204	.008
	54	3	2	0.285	.008
	62	3	2	0.615	.021
	64	3	2	0.277	.018
	65	4	2	0.256	.012
Group 2. unfested	6	1	30	0.193	.009
	8	1	32	0.237	.031
	20	1	38	0.234	.044
	11	2	29	0.982	.044
	2	2	38	0.156	.010
Control negative				0.090	.011

* Absorbance value of color reaction at 10 min (mean of 3 replicates).
† Numbering from the 1st day of infestation. The infestation terminated on the 4th day.

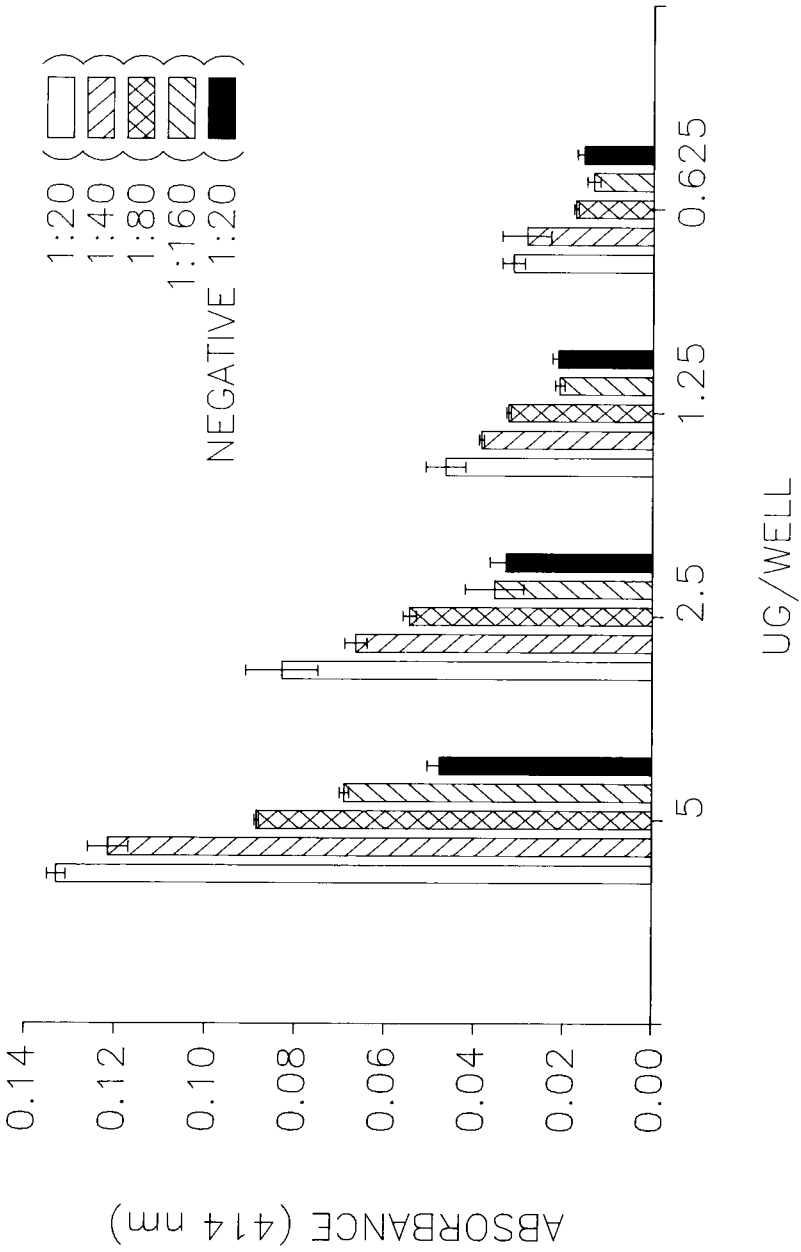

Fig. 1. Block titration to determine optimum SWl antigen concentration and sheep serum dilution for dry plate field ELISA. SWl antigen concentration in ug/well. Two replicates each sample.

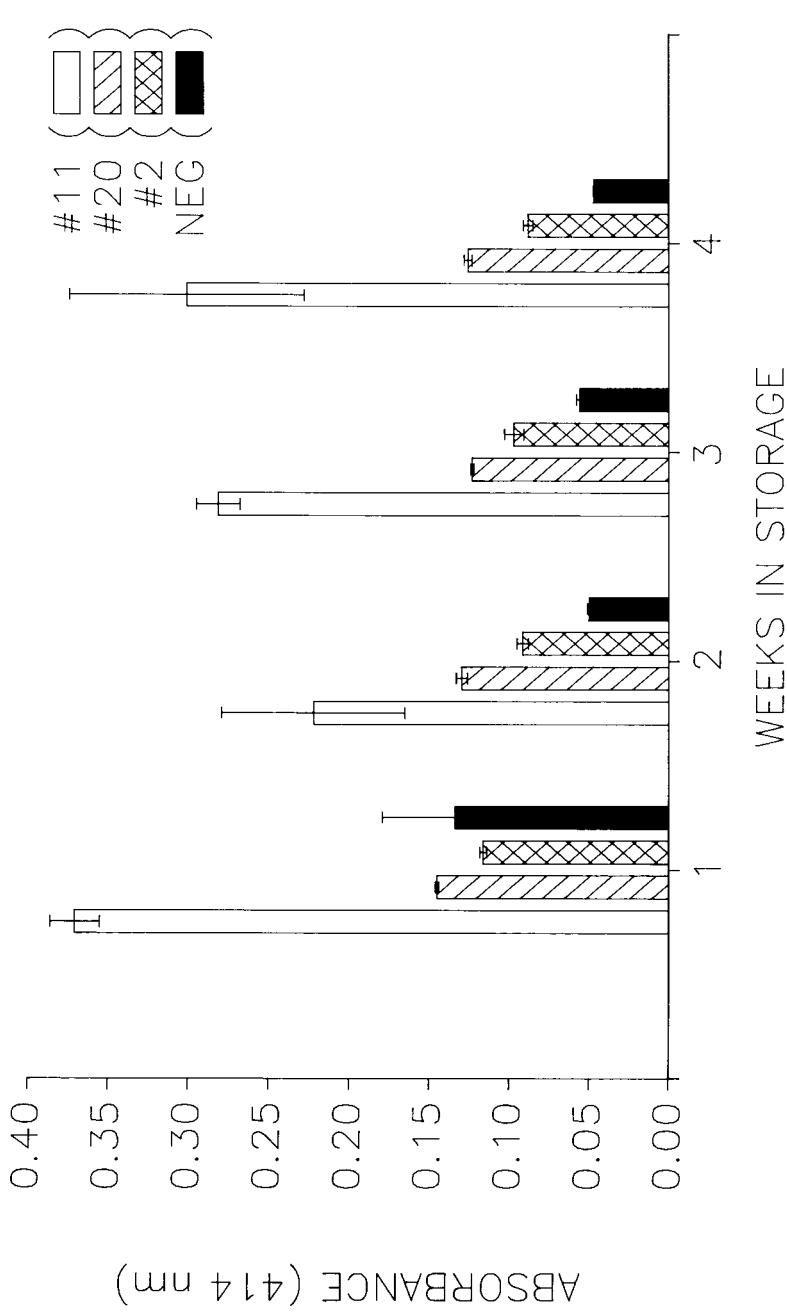

Fig. 2. Stability of SWI antigens and reliablity of anti-screwworm antibody dectection in sera with high (#11), medium (#20), and low (#2) antibody concentrations using dry field ELISA microtiter plates sensisized with SWI antigens and stored 1-4 weeks at room temperature (23°). Positive and negative sera diluted 1:20. Two replicates each sample.

Discussion

Laboratory reared screwworms are fed a gel media that contains bovine blood proteins. In the preparation of SWl antigens from 3rd-instar screwworm larvae, there was concern that occult bovine blood proteins could cause high background reactivity in the ELISA as a result of nonspecific binding of immunoreagents. Background activity was low and serum samples containing anti-SWl antibody could be distinguished qualitatively from negative control serum. These results suggested that further purification of SWl protein for the purpose of removing any occult proteins was not required. This report is the first to demonstrate specific antibodies to antigens of screwworm in the serum of an infested host.

An ELISA (field ELISA) was developed which could be used in the field to qualitatively determine if an animal had been recently exposed and/or infested with screwworms. Future studies to define the limits of detection would include the kinetics of antibody development and decline with a single infestation of variable numbers of larvae. A dry plate configuration for protein binding was chosen over a wet plate configuration for reasons of protein stability. Conditions in the field, particularly in the tropics, could affect storage of these proteins. Protein degradation in a wet plate as a result of bacterial growth or inherent proteolytic activity could destroy the antigenicity of the bound proteins and reduce the shelf-life of the activated microtiter plate. Dry SWl antigens bound to microtiter plates were found to maintain their antigenicity for a minimum of 4 weeks at room temperature. In addition, the test was reliable for the detection of an infested sheep with a low concentration of anti-SWl antibody for the same time period. Any loss of antigenicity as a result of storage would have the greatest effect upon detection of animals with low concentrations of antibody, leading to a false negative determination. These results suggest that the test can be taken to the field without concern for antigen stability over this time period.

Absorbance values (color development) were amplified by shaking the microtiter plate. If access to electricity or a plate shaker is not convenient, incubation times can be extended or the contents of the wells agitated by pipeting. A spectrophotometer was used in this study to read the developing color reaction. In the field, the developing color reaction can be visually scored as qualitatively positive or negative or, on an arbitrary numerical system, relative to positive and negative controls.

Residual anti-SWl antibody was detected in the serum of all convalescent sheep. This included a sheep with a single previous infestation that was bled 38 days after 3rd-instar larvae were removed from the wound. This sheep was exposed to SWl proteins for only 4 days of actual infestation by living larvae. Although soluble SWl proteins could remain in the recovering wound, the data suggest that SWl antigens are highly immunogenic in the sheep.

The field ELISA could find application for survey and detection of noninfested susceptible hosts previously exposed to screwworms. Preparation of species specific enzyme-linked second antibody conjugates will allow the screening of a wide variety of animal species. Presently, the potential for cross reactivity with antigens of other myiasis causing fly larvae is unknown. If cross reactivity proves to be a problem, purification of specific srewworm antigens will be necessary. Purification of specific antigens is expected to further increase the sensitivity and enhance the specificity of the assay.

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References Cited

Borgstrom, F. A. 1938. Studies on experimental *Cochliomyia americana* infestations with special reference to the bacterial flora and the development of immunity. Am. J. Trop. Med. 18: 395-411.

Bradshaw, L. J. 1966. Introduction to Molecular Biological Techniques. Prentice-Hall, Englewood Cliffs, NJ. 107 pp.

Laake, E. W., and C. L. Smith. 1939. The development of immunity in calves to the larvae of *Cochliomyia americana* C. & P. J. Encon. Entomol. 32: 339-342.

Lindquist, A. W. 1937. Myiasis in wild animals in southwestern Texas. J. Econ. Entomol. 30: 735-740.

Mathews, H. M., K. W. Walls, and A. Y. Huong. 1984. Microvolume kinetic-dependent enzyme-linked immunosorbent assay for amoeba antibodies. J. Clinic. Microbiol. 19: 221-224.

O'Donnell, I. J., P. E. Green, J. A. Connell, and P. S. Hopkins. 1980. Immunoglobulin G antibodies to the antigens of *Lucilia cuprina* in the sera of fly-struck sheep. Austral. J. Biol. Sci. 33: 27-34.

Sandeman, R. M., V. M. Bowles, I. N. Stacey and P. R. Carnegie. 1987. Acquired resistance in sheep to infection with larvae of the blowfly, *Lucilia cuprina.* Int. J. Parasitol. 16: 69-75.

Taylor, D. B., and R. L. Mangan. 1987. Comparison of gelled and meat diets for rearing screwworm larvae, *Cochliomyia hominivorax* (Diptera: Calliphoridae). J. Econ. Entomol. 80: 427-432.

Thomas, D. B. 1987. Incidence of screwworm (Diptera: Calliphoridae) and torsalo (Diptera: Cuterebridae) myiasis on the Yucatan Peninsula of Mexico. J. Med. Entomol. 24: 498-502.

Voller, A., D. Bidwell, and A. Bartlett. 1980. Enzyme-linked immunosorbent assay. *In* N. R. Rose and H. Friedman [eds.], Manual of Clinical Immunology, 2nd ed. Am. Soc. Microbiol. Washington, D.C.