The Use of Blood-Fed Mosquitoes as Diagnostic Tools for the Detection and Monitoring of Infectious Disease in Wildlife

by

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ABSTRACT

When a mosquito bites its host, it carries away a blood sample containing specific antibodies which can provide a history of the immune responses of the vertebrate host. The purpose of this research was to determine whether antibodies which are specific for agents of infectious disease could be detected in blood-fed mosquitoes. Mosquitoes were fed on blood containing a specific antibody and assayed using an enzyme-linked immunosorbent assay to determine the limits of detection of the antibody post-feeding over time, and at different temperatures and antibody concentrations. The results showed that the antibody, at an initial concentration of 1 μg/ml could be detected in mosquitoes for 24-72 hours after feeding. Blind tests simulating the assay of feral mosquitoes detected positive mosquitoes with few false negatives and no false positives. This research indicates that the collection and immunological assay of mosquitoes could be used to detect and monitor infectious disease in wildlife.

DEDICATION

To my wife, Rosanna and my children, Christina and David for all their love and understanding and to my parents and to Rosanna's parents for all their support. Family makes the best team.

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1 INTRODUCTION

Human health and livestock production may be impacted by infectious diseases of wildlife living close to human habitations and livestock operations. Expanding development, intensive farming practices, the translocation of animals and the adaptation of wildlife to urbanized environments have increased the exposure of humans to zoonotic diseases (diseases transmitted from animals to humans) such as hantavirus, Lyme disease, avian influenza, and rabies. Many diseases that affect livestock may originate in neighboring wildlife. For example, bovine tuberculosis, brucellosis, anaplasmosis and prion diseases can be transmitted to livestock from wild ruminants (Kistnel 1982). Poultry production may be affected by fowl pox, Newcastle disease or avian influenza that has been transmitted from wild birds (Palmer and Trainer 1971). The ability to detect and monitor disease in wildlife is essential to understand and possibly prevent the transmission of these diseases to humans and livestock.

Infectious diseases have become a major concern in the conservation of animal species already threatened by habitat loss and exploitation. Population declines in many wildlife species have been associated with disease. For example, as their habitats are disrupted, the great apes, including gorillas, chimpanzees and orangutans are being impacted by herpesviruses (Sakulwira et al. 2004), hepatitis B (Sall et al. 2005), dengue-2 virus, Japanese encephalitis, malaria (Wolfe et al. 2001, 2002; Kilbourn et al. 2003) and Ebola haemorrhagic fever (Feldmann et al. 2004).

Steller's sea lions (*Eumetopias jubatus*) have experienced recent and dramatic population declines and it has been postulated that infectious disease may have played a causative role. A number of disease agents occur in marine mammals within the habitat range of Steller's sea lions including morbilliviruses, influenza virus, phocine herpesviruses, calciviruses, *Leptospira* spp., *Brucella* spp, *Chlamidia psittaci*, *Toxoplasma* spp. as well as a number of nematode species including *Anisakis* spp. Any of these pathogens could produce epizootics or reduce sea lion reproduction (National Research Council 2003). These threats have spurred demands for systematic monitoring for disease in Steller's sea lions (Burek et al. 2003).

Surveillance of disease in wildlife commonly involves trapping or killing large numbers of animals for direct sampling of blood and tissues. This can be difficult, expensive and dangerous to field personnel, both in the handling of animals and in the risk of exposure to disease. Sampling of wildlife may be unacceptable in parks and wildlife preserves, and capture and invasive sampling methods may be inappropriate with endangered species. Therefore, benign testing methods are preferred (Mörner et al. 2002).

Blood-fed mosquitoes that are captured in the wild contain a sample of blood taken from vertebrate hosts. Whole blood includes specific immunoglobulins (Ig) which, if detectable, can provide a history of the immune responses of the vertebrate host. If these antibodies, specific to particular disease agents, could be detected in blood feeding arthropods, then the collection and immunological assay of mosquitoes or other haematophagus arthropods, could be used to detect and monitor these diseases in wildlife, reducing the need to capture or kill animals and providing a non-invasive and non-disruptive means of monitoring wildlife health.

Immunoglobulins make up 20% of plasma protein (Edman 1970). In normal serum, γ globulin (IgG) is more than 70% (7-15 mg/ml) of the Ig fraction and is the most stable Ig class with a half life of 23 days in the serum (Guttmann 1983). The serum concentration of IgG to a specific antigen can vary from 1 pg/ml – 10 mg/ml (Harlow and Lane 1999).

IgG in the blood regularly passes through the walls of small venules and is the only Ig class that can cross the placenta (Atlas 1984). It also can pass through the arthropod gut into the haemolymph (Brossard and Rais 1984; Minoura et al. 1985; Pruett and Thomas 1986; Vaughan and Azad 1988; Vaughan et al. 1998).

To develop detection and monitoring systems using mosquitoes, several factors must be considered: **1.** The ability to detect specific antibodies in very small blood samples. **2.** The effects and rates of digestion of host antibodies in the blood meal that would limit the time, after the blood meal, that specific antibodies could be detected. **3.** Identification of the host species (blood source). **4.** The best methods of collecting blood-fed mosquitoes in the field. These topics are considered below.

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1.2 Arthropods and Antibodies

The presence of host antibodies in the blood meals and haemolymph of arthropods has been studied as a means of controlling pest mosquitoes and other haematophagus arthropods. If host animals are immunized with vector arthropod tissues such as midgut, head or whole body homogenate, host antibodies to these antigens may

affect the fecundity (Sutherland and Ewen 1974; Balashov 1984) or survival (Alger and Cabrera 1972) of arthropods that subsequently feed on their blood.

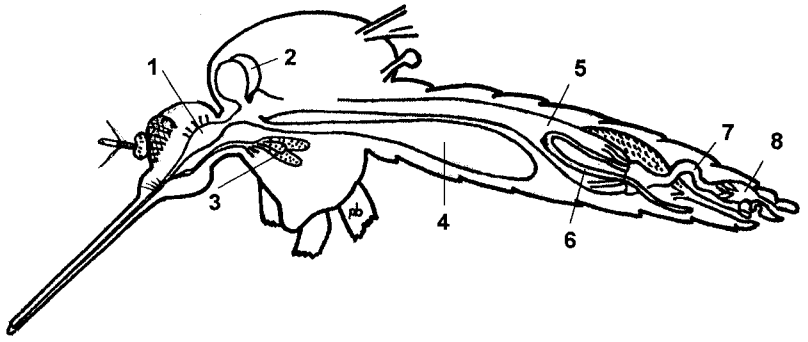
Studies examining the possibility of vaccinating against arthropod vectors have demonstrated that specific host antibodies persist and can be detected in arthropod blood meals, in the haemolymph and bound to gut epithelia (Nogge and Giannetti 1980; Ackerman et al.1981; Minoura et al. 1985; Hatfield 1988; Lackie and Gavin 1989).

Fujisaki et al. (1984), using immunofluorescence, was able to detect specific antibodies to the pathogenic piroplasm, *Theileria sergenti* in the haemolymph of ticks that had fed on experimentally infected calves, and Vaughan and Azad (1988) using a similar technique, detected antibody to *Rickettsia typhi*, the agent of murine typhus, in blood meals of 5 species of mosquitoes that had fed on experimentally-infected rats. In both of these studies normal, pathogen-specific antibody concentrations found in blood were detectable 24-48 hours after the blood meal was taken.

Hatfield (1988) found that specific antibodies were present in the haemolymph of *Aedes aegypti* and retained their immunological properties up to 48 hours after feeding. Host antibodies appear in the mosquito's haemolymph shortly after ingestion and this is believed to be due to the slow formation of the peritrophic matrix, a structure secreted by the midgut epithelium that encapsulates the blood meal in the midgut. Lackie and Gavin (1989) reported persistence of specific host antibodies in *Anopheles stephensi* whole-body homogenates up to 9 days after feeding and found that the antibody was in the haemolymph after it disappeared from the gut (see Figure 1). These studies of host

antibody persistence in the blood meal and tissues of arthropods were aimed at developing systems

Figure 1. Anatomy of the mosquito digestive system. 1. Pharyngeal pump. 2. Dorsal divercula. 3. Salivary glands. 4. Crop. 5. Midgut. 6. Malpighian tubule. 7. Intestine. 8. Rectum. Diagram courtesy of Dr. Peter Belton.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for the purpose of controlling vector arthropods, but the data also support the practicality of using of host antibodies in arthropods to detect infectious disease. Most disease detection systems that use arthropods detect the presence of pathogenic organisms in the blood meal by culture methods (eg. Borrelia), or use serological techniques to detect antigens of pathogens in the blood meal. Some systems detect arthropod specific stages of a pathogen (eg. *Plasmodium*). The presence of host antibodies in blood feeding arthropods has not been used for detection or monitoring disease. This study explores the feasibility of adapting existing technology for this purpose.

Host antibodies that persist in the blood meals and haemolymph of haematophagus arthropods have been detected using various serological techniques including immunoprecipitation (Fujisaki et al. 1984; Minoura et al. 1985,) immunofluorescent antibody technique (Schlein et al. 1976; Ackerman et al. 1981; Fujisaki et al. 1984; Vaughan and Azad 1988) radioimmunoassay (Ben-Yakir; 1989) and Enzyme-Linked Immunosorbent Assay (ELISA) (Ben-Yakir et al. 1987; Hatfield 1988; Lackie and Gavin 1989; Vaughan et al. 1998). Of these techniques, ELISA is considered to be the most specific, sensitive and useful in the field (Burkot et al. 1981; Service et al. 1986; Beier et al. 1988). The sensitivity of ELISA can be greatly enhanced by the use of monoclonal antibodies or antibody fragments (Ishikawa 1999). ELISA was selected for the detection of antibody because it is simple and economical to use, can be used in the field, and can handle large numbers of samples. ELISA was chosen for these reasons.

There are a number of types of ELISAs. The indirect ELISA is the simplest type of microplate ELISA (Kemeny 1991), in which microplate wells are coated with antigen and then the test samples are added to the antigen coated wells. Specific antibodies in the

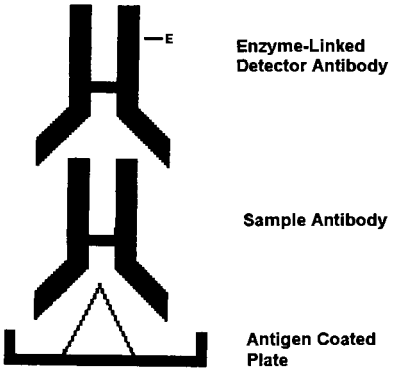
sample bind to the antigen and the bound antibody is detected by a second, enzyme-labeled antibody specific to the bound antibody.

1.3 The Mosquito Blood Meal

The blood ingested by mosquitoes provides nutrients, primarily proteins, for egg production and energy for the mosquito. Immunoglobulins make up 20% of the plasma protein of blood (Edman 1970) and while some immunoglobulin can pass into the haemolymph of the mosquito, most of it likely is digested with the blood meal (Hatfield 1988). The size of the blood meal and the rate at which it is digested are important factors in the detection of specific antibodies in the blood meal. Digestion rate is affected by a number of factors, particularly the temperature of the blood meal in the midgut. Digestion rate also limits the time for the identification of the blood meal source.

Collecting mosquitoes while they contain an undigested blood meal and properly storing and transporting these mosquitoes are important factors in developing a detection system.

Figure 2. An indirect enzyme-linked immunosorbent assay


—E
Enzyme-Linked
Detector Antibody
Sample Antibody
Antigen Coated
Plate

1.3.1 Blood meal volume

The size of the blood meal varies between species of mosquito, particularly in proportion with the body size. *Aedes aegypti* blood meal volumes have been recorded from 2.2-7.0 µl (Klowden and Lea 1979) and the volume is correlated with female size (Briegel 1990). *Aedes sollicitans* has been recorded with blood meals as large as 11 µl (Clements 1992).

Anopheline mosquitoes cannot grossly distend their midgut and abdomen as other mosquitoes can and discharge as much as 55% of the blood fluids though the anus, a process known as prediuresis. Mean retained blood meal volume after prediuresis for *Anopheles albimanus* was 1.5µl and blood proteins are concentrated in the retained meal (Clements 1992).

Vaughan and Azad (1988) tested 5 species of mosquitoes including, *Anopheles stephensi, An. gambiae, An. albimanus, Aedes aegypti* and *Culex pipiens* for specific IgG after feeding them on seropositive rats. They found that mosquitoes that stayed on the host for extended periods and underwent prediuresis, had detectable amounts of IgG into the haemolymph, however, IgG was less likely to be found in mosquitoes that took smaller blood meals and did not undergo prediuresis.

In the wild, mosquito blood-meal volumes may often be less than the mosquito's capacity if feeding is interrupted by the defensive behaviors of the host (Edman and Kale 1971; Edman et al. 1974). Klowden and Lea (1979) demonstrated that blood meals were consistently smaller in mosquitoes that had fed on unrestrained hosts compared to those that had fed on restrained hosts.

1.3.2 Digestion of the blood meal

Digestion rates and environmental conditions, particularly temperature and variability in digestion rates between mosquito species, are important factors to consider in using mosquitoes for antibody studies. Houk and Hardy (1982) defined five distinctive phases to blood meal digestion in *Culex tarsalis*: "0-4 hr – osmoregulation; 4-8 hr – induction of synthesis of secretory materials; 8-24 hr – synthesis and secretion of synthesized materials (peritrophic membrane and digestive enzymes); 24-36 hr – digestion and transport of blood meal across the midgut epithelium into the haemocoel and beginning at 48 hr – resting stage." These phases are similar in most blood feeding mosquitoes but may vary in timing between mosquito species, with temperature and may be different in mosquitoes that use prediuresis to concentrate the blood meal and those that do not.

After feeding, the blood meal is held in the mosquito midgut where digestion takes place. The midgut's functions include synthesis and secretion of digestive enzymes and the peritrophic matrix, and the absorption of nutrients. After water, proteins are the main component of blood, but blood also contains carbohydrates and lipids and the mosquito midgut secretes proteinases, carbohydrases and esterases (Clements 1992).

Ae. aegypti females have at least eight different proteinases and seven of these are trypsin-like enzymes (Kunz 1978) which account for 75% of the proteolytic activity in mosquito gut homogenates (Briegel and Lea 1975) and trypsins are the main proteolytic enzymes for blood digestion (Yang and Davies 1971). Trypsin accounts for

75% of enzyme activity in mosquito faeces, where it is present at half of the level found in the midgut at full activity (Briegel 1975).

The production of gut proteinases, is induced by the increase in globular protein concentration in the midgut and reaches a peak about 20 hours after feeding at 27°C. The rate of digestive enzyme synthesis and the kinetics of enzyme reactions are affected by temperature: Proteolytic activity peaked in half the time at 32°C compared to 22°C in *Ae. aegypti* (Briegel and Lea 1975) and this directly affects the rate of digestion of the blood meal. For example, tropical mosquitoes can digest a blood-meal in 24-48 hours whereas it can take longer than a week in temperate areas (Service et al. 1986). Hibernating females of *Culiseta annulata*, kept at 6-8°C took 28-31 days to digest a blood meal (Clements 1992).

The time needed for a mosquito to digest a blood meal varies a great deal with temperature but when the temperature is near the optimal digestion temperature for the mosquito species, it is generally a positive non-linear relationship based on enzyme kinetics and the timing and rate of enzyme secretion (Hatfield 1988; Billingsley and Hecker 1991). Arthropods such as fleas which feed constantly have very rapid digestion rates and digestive enzymes are synthesized continuously (Vaughan et al. 1998) but in the intermittently-feeding mosquitoes digestive enzymes are not present until the blood meal is taken. The digestion rate is initially slow and accelerates as synthesis of enzymes is induced and rises to its peak.

The peritrophic matrix may serve as a solid support for digestive enzymes (Walker et al. 1980; Jacobs-Lorena and Oo 1996). Immobilized enzymes would be more efficient,

as they are retained in the gut for the duration of digestion and not excreted. Digestion begins at the periphery of the blood meal within the ectoperitrophic space and trypsin activity is higher at the posterior end of the midgut. Most of the enzyme activities are on the outer surface of the blood mass but trypsins can penetrate the peritrophic membrane (Graf et al. 1986).

Digestion in *Ae. aegypti* is also affected by age, mating and parity and may be controlled by hormones. Downe (1975) demonstrated that the digestion rate was higher in mated females or in females that had been injected with an extract of male accessory organs known as matrone. In nature, matrone is injected into females during mating rendering them unreceptive to further fertilization. In unfed females, matrone may induce host seeking behavior (Clements 1992). The female stores enough sperm to fertilize a number of egg batches (Clements 1992). This means that most blood-fed females are already mated. Some virgin females do not develop eggs after a blood meal as fast as mated females (Clements 1992).

Anopheline mosquitoes actively excrete blood meal fluids (prediuresis) while feeding. This behavior concentrates the blood proteins that are retained and may be a significant factor in detecting specific antibodies. Few data are available for the concentration of proteins in the anopheline gut, but erythrocytes are concentrated in the midgut at factors of 1.85 for *Anopheles gambiae*, 1.39 for *Anopheles arabiensis* and 1.23 for *Anopheles dirus* (Clements 1992).

The fate of IgG in the ingested blood meal is not clear, but it is probably broken down by digestive enzymes. When the blood components were examined using

immunoblot analysis during the digestion process in *Ae. aegypti*, intact proteins disappeared 24-48 hours after ingestion (Billingsley and Hecker 1991). Nevertheless, albumin and IgG were the most persistent proteins (Irby and Apperson 1989). Yeates (1980) reported that crude extract from the posterior midgut of *Ae. aegypti* was almost inactive with IgG. The IgG molecule can be cleaved into its two specific binding fragments Fab and $F(ab')_2$ and its Fc fragment by enzymes. The two Fab fragments are still capable of binding to specific antigens after cleavage (Harlow and Lane 1999) and should be detectable in an ELISA. If the detector (secondary) antibody in the ELISA is specific to the Fc region of the IgG it will detect only intact antibodies (Vaughan et al. 1998) as the Fc fragment alone cannot bind to the antigen. Thus, digestive processes could possibly denature the IgG protein causing it to lose its binding properties and release the Fc fragment thereby making it undetectable by serological assay.

1.3.3 Blood meal identification

An important factor in using mosquito blood meals for infectious disease surveys is the need to identify the host that provided the blood meal. A number of techniques have been applied successfully to host identification. Immunological methods to identify blood meals include the use of the precipitin test which was first devised by Uhlenhuth (cited in Bates 1949), first applied to mosquito blood meals by Bull and King (1923) and improved for testing large numbers of mosquitoes by Rice and Barber (1935). Several variations of the precipitin method are discussed by Templis (1975) and mosquito blood meal identification techniques are reviewed by Washino and Templis (1983). Edman and Schmid (1970) were able to identify blood meals using the precipitin method

with blood fed mosquitoes after they had been dried and stored at low humidity for 4 years.

An alternative technique originally suggested by Amantea (1926) using the crystallization patterns of hemoglobin on glass to identify hosts was tested by Washino and Else (1972). Other methods include fluorescent antibody technique (McKinney et al. 1972), passive hemagglutination inhibition technique (Weitz 1963; Boreham 1975) and the Enzyme-Linked Immunosorbent Assay (ELISA).

The ELISA technique was tested for host identification of mosquito blood meals by Burkot et al. (1981) and by Service et al. (1986) and was found to be a very sensitive technique giving good results even with very small blood meals. The technique could be used in the field with minimal training and laboratory support. Hosts could be identified using ELISA with as little as 0.02 µl of blood (Service et al. 1986). Mosquito hosts are now routinely identified using Polymerase Chain Reaction (PCR) (Gokool et al. 1993; Boakye et al. 1999; Ngo and Kramer 2003) and individual human hosts can be identified using DNA fingerprinting after using PCR to amplify DNA fragments from mosquito blood meals 10 hours after blood feeding (Coulson et al. 1990; Michael et al. 2001; Ngo and Kramer 2003). PCR provides much more specific identification about the host but serological techniques are more economical when only the identification of the broad taxonomic group of the host is needed.

Service et al. (1986) using ELISA, demonstrated that the hosts of *Ae. aegypti* could be identified in all engorged and in half of gravid mosquitoes up to 26 hours post-feeding at 24°C and in half of mosquitoes that had only a trace of blood meal remaining.

The stage of ovary and egg development or gonotrophic state of the mosquito can be a useful measure of the post-feeding time (Service et al. 1986).

Identification of the host species may be complicated by mosquito and host behavior, particularly with multiple biting, involving different host individuals or species within a single gonotrophic cycle. When mosquito feeding was compared between restrained and unrestrained hosts, the blood meals were consistently smaller in the mosquitoes that had fed on unrestrained hosts (Klowden and Lea 1979). The defensive behavior of hosts may often cause *Culex* mosquitoes to complete their blood meal on a second host (Anderson and Brust 1997). Multiple blood meals in other culicine mosquitoes appear to be caused by interrupted feeding (Briegel and Horler 1993; Edman and Scott 1987).

Anopheline mosquitoes may take multiple blood meals before maturing eggs and the first blood meal is used for energy and reserves. Multiple feeding may be adaptive for anophelines as they function close to their energetic minimum (Briegel and Horler 1993; Anderson and Roitberg 1999). The frequency of multiple feeding in wild mosquitoes varies from 0-50% in anopheline and culicine mosquitoes (Wekesa et al. 1997).

1.3.4 Collection of blood fed mosquitoes

To collect mosquitoes with usable blood meals, they must be captured before it is digested completely. After blood feeding, female mosquitoes generally enter a resting stage in which they find a secluded hiding place to avoid predators while they digest the

blood meal and develop their eggs (Service 1976). This can make collection of resting mosquitoes challenging.

A number of general trapping methods have been developed. The New Jersey trap and its successor, the CDC trap, have a light source and a down-draft electric fan that attracts and sucks mosquitoes into the trap (McNelly 1989). A new design known as a counter-flow geometry trap has been tested and has been reported as a much more effective overall trap compared to the basic down flow designs (Kline 1999). These traps are only effective in collecting flying mosquitoes. Other trapping methods using bait animals, CO_2 and other attractant chemicals such as 1-octen-3-ol (Kline 1994) are most effective with actively host-seeking mosquitoes but may repel blood-fed females. However, an improved ratio of blood fed mosquitoes was reported with the addition of CO_2 sources such as dry ice or bottled CO_2 to the CDC traps (Newhouse et al. 1966; Morris and DeFoliart 1969; Feldlaufer and Crans 1979) but if these mosquitoes are actively flying they may be only partially fed. The removal of the light from the trap may reduce the problem of capturing other insects that complicates sorting and makes traps less conspicuous (McNelly 1989). If blood-fed mosquitoes are flying it is light that would most likely attract them. Oviposition traps may only attract gravid mosquitoes that have already digested their blood meal (Moore and Gage 1996).

A number of other techniques have been developed for collecting resting mosquitoes including sweep netting in vegetation, the use of suction devices that can be used in tree hole, animal burrows, crevices and caves, and the use of artificial resting shelters which can be effective as traps. These shelters are attractive to blood fed mosquitoes as hiding places. Various types of box and fabric shelters have been used.

They are usually painted black or red and are more effective if they open away from the morning sun (Service 1976). Resting mosquitoes can be collected from these shelters with nets, suction devices or by the use of anesthetics or pesticides.

In an experiment comparing plywood resting boxes and fiber pots for mosquito collection, Komar (2003) found that fiber pots were as effective as unshielded resting boxes at attracting *Culiseta melanura* and that 16-31 % of the *C. melanura* females in the fiber pots were blood engorged compared to 18-20% in the resting boxes.

Blood-fed mosquitoes that have been collected should be assayed immediately or should be frozen for storage and transported on dry ice. Alternate methods may be desiccation of the mosquitoes by killing them and placing them in 1.5ml ventilated tubes over silica gel (Bangs et al. 2002) or, for short term storage, holding them alive at the lowest temperature that they can survive to slow digestion of the blood meal.

In summary the mosquito blood meal and the mosquito provide a package of information about the host including its identity and its immunological status as well as information about the biting habits of the mosquito species. This could be of great value in disease surveillance programs. This information can be made accessible through modern laboratory methods such as those described above. In the next section I hypothesize that antibodies in mosquito blood meals can be detected using ELISA and that this method could be applied to the diagnosis and monitoring of disease in wildlife.

2 METHODS AND MATERIALS

I used an enzyme-linked immunosorbent assay system and conducted experiments to examine the fate of a monoclonal antibody ingested in artificially-fed blood meals by two species of mosquitoes. The ultimate goal is to sample feral, blood-fed mosquitoes for specific host antibodies in a disease surveillance program.

The objectives of this study are to:

1. Determine the limits of detection for specific IgG in mosquito blood meals using an artificial antibody-antigen system using the concentration range normally found in infected mammals.

2. Develop a simple enzyme-linked immunosorbent assay system that could be adapted for use in field research.

3. Evaluate the challenges of developing a monitoring system using these techniques

2.1 Mosquitoes and Rearing

Aedes aegypti (Black-eyed Liverpool strain) were maintained though their life cycle in an incubator at 29°C with 12:12 L:D photoperiod and 60-70% relative humidity. *Aedes togoi* larvae were collected from marine rock pools near Gibsons, British Columbia and reared through 5 generations in an incubator at 20°C with 16:8 photoperiod and 70% relative humidity. Larvae of both species were reared on ground TetraMin® fish

food and the adults were maintained on 10% sucrose solution provided in cotton balls. *Aedes togoi* adult diet was supplemented with raisins.

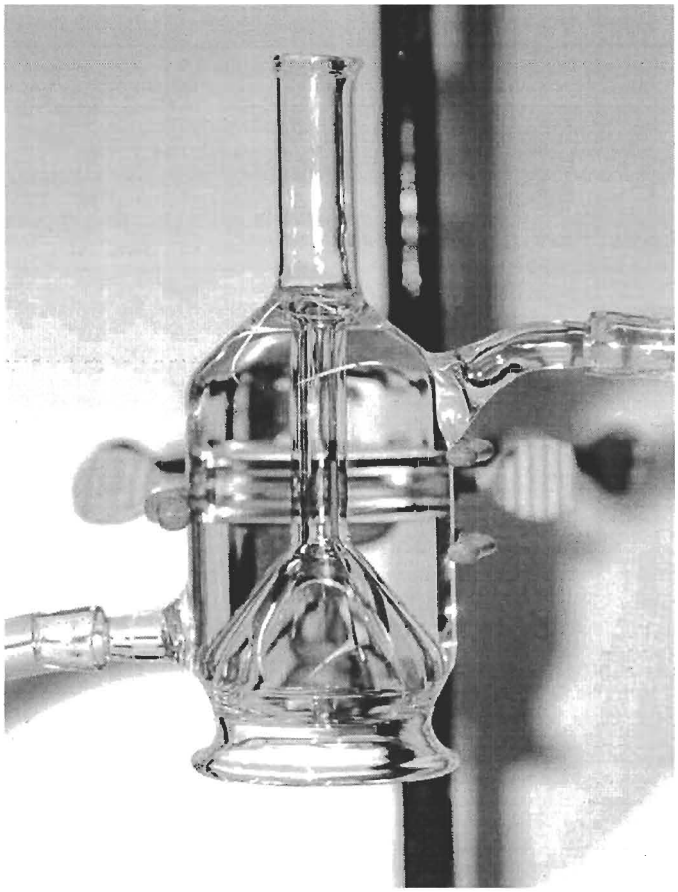
Ae. aegypti were chosen because they are a well-studied species and there is a considerable amount of information available on digestion and blood feeding in this species. Much less is known about *Ae. togoi* but it was chosen because it is a local coastal species and may be of interest in the study of infectious disease in humans, marine mammals and seabirds.

2.2 Feeding

Mosquitoes were fed on an artificial feeder consisting of a funnel-shaped glass tube with a water jacket that was heated to 37°C by a circulating water bath. A piece of Parafilm® was stretched across the mouth of the tube and blood was placed onto the upper surface of the membrane. *Ae. aegypti* held in paper cups with a mesh top were able to feed through this membrane from beneath the feeder. *Ae. togoi* were fed by fitting the feeder to a large mesh cage containing the mosquitoes (Figure 3).

After removing the cotton balls with the sucrose solution for 24 hours, mosquitoes were fed on citrated human blood from the author to which, a mouse monoclonal anti-chicken egg albumin antibody (MAb) (Sigma) had been added. This MAb, an IgG1 class Ig was diluted from an initial concentration of 10mg/ml to a 1:10 dilution in phosphate-buffered saline with Tween-20 (PBS-T) in working aliquots and stored frozen. For feeding, the MAb was diluted in blood to a final dilution of 1:1,000 (10 μg/ml) or 1:10,000 (1 μg/ml).

Figure 3. Artificial feeder for mosquitoes. A circulating water bath provides 37°C water which is circulated through the water jacket. Mosquitoes feed on blood through a membrane at the bottom.



Mosquitoes were allowed access to the blood for 30 minutes. All fed mosquitoes were held in incubators for specified times at controlled temperature, photoperiod and humidity and then were stored frozen at -84°C. Before being assayed, the mosquitoes were ground with a plastic tissue grinder in 1.5ml tubes in 200µl of PBS-T, centrifuged at 16,000g for 3 minutes at 4°C and the supernatant used in the ELISA.

2.3 Controls

A sample of blood containing the antibody was retained for use as a positive control in each ELISA. This sample was held in a 37°C water bath while the mosquitoes were fed. Negative controls included blood without the antibody, an isotype mouse IgG1 control at a 1:1000 dilution in PBS-T, and PBS-T blanks that were used to zero the plate reader. The blood controls were frozen at -84°C. The isotype mouse IgG was used to determine the background levels for the assay. The positive and negative control blood was diluted to 200µl in PBS-T before the assay. The amount of blood in this dilution was based on the average blood meal size estimated from a sample of mosquitoes from the same cohort, weighed before and after the blood meal for each assay.

2.4 ELISA

Ninety-six well plates (Nunc Maxisorb®) were coated with the antigen - 10 µg/ml chicken-egg albumin (Sigma) (200µl/well) and incubated at 37°C for 30 minutes. The solution was removed and the plates washed 3 times with PBS-T. PBS (200µl)

containing 3% non-fat milk was used to block the plates to reduce non-specific binding of proteins to the plate. The plates were frozen with the blocking solution at -18°C until used.

Checkerboard titrations (two-dimensional serial dilutions) were run to determine the optimal working concentrations of the primary and detection antibodies and to develop a reference dilution series to calibrate the assays. The reference dilutions of the primary antibody ranged from 1:100,000 – 1: 3,200,000, halving the concentration (1:2) with each dilution.

When the samples and controls were ready, and the plate had been thawed and washed 3 times with PBS-T, 200µl of each sample and control was added to the plate and incubated for 2 hours at room temperature (all samples and controls in triplicate). The plate was then emptied and washed 3 times with PBS-T.

Goat anti-mouse IgG (whole molecule) (Sigma) antibody which was conjugated with alkaline phosphatase was used as the detector antibody at a 1:1000 dilution. 200µl of the detector antibody was added to each well and the plate was incubated for 2 hours at room temperature. The plate was emptied and washed 3 times with PBS-T and then 200µl of the enzyme substrate (Sigma Fast® p-nitrophenyl phosphate tablets dissolved in distilled water) was added to each well and the colour was allowed to develop for 30 minutes. The development was stopped by adding of 50µl of 3 M NaOH and the plates were read on a Bio-Tek EL340 Automated Microplate Reader (Bio-Tek Instruments Inc. Winooski, Vermont) at 405nm. The plate reader was zeroed on the PBS-T blank wells and the reading from the non-specific mouse isotype IgG wells provided a background

reading which was subtracted from the other readings. Based on back-ground readings in preliminary assays, the lower limit for positives was set at OD = 0.050 which is 3 times the back-ground level.

Preliminary assays tested the polyclonal detector (secondary) antibodies that were specific just to the Fc region and to the whole IgG molecule of MAb. Both antibodies appeared to work equally well but the whole IgG molecule-specific antibody was chosen for the experiments because cleavage of MAb may take place during digestion and the Fc fragments may be lost. Vaughan et al. (1998) reported that detection using the Fc specific detector antibody suggests that the detected IgG is intact.

2.5 Experiment 1. Detection at Two Concentrations

This experiment examined the effect of MAb concentration on the detection of MAb over selected time intervals. The two concentrations had a 10 fold difference and were both within the normal range of specific IgG in seropositive mammals (Harlow and Lane 1999). The time intervals were based on preliminary tests and the expected time frame for digestion established by other researchers.

Fifty individual *Ae. aegypti* were fed on one of two concentrations of MAb in blood; 1:1000 and 1:10,000 corresponding to 10μg/ml and 1 μg/ml, respectively (mean blood meal size = 1.65 μl, n = 5). After feeding, the mosquitoes were chilled to inactivity for 1minute at -20°C and blood-fed insects were randomly divided into groups of 3 and put into containers and held for 1, 6, 12, 24 and 48 hrs at 29°C and 60-70% relative humidity. A sugar solution (10% sucrose) was available *ad libitum*. After each time

interval the designated mosquitoes were again chilled to inactivation, placed in 1.5 ml tubes and frozen at -84°C until assayed (Figure 4).

2.6 Experiment 2. Detection at Two Temperatures

To examine the effects of temperature on the putative digestion of MAb and on the post-feeding duration of detection, two groups of mosquitoes were fed MAb in blood and held at two temperatures over selected time intervals.

Fifty individual *Ae. aegypti* were fed 1 μg/ml MAb in blood (mean blood meal size = 2.0 μl, n = 5), fed mosquitoes were randomly divided into two groups, placed in two holding treatments, one at 29°C and 60-70% humidity and one at 20°C and 70% humidity. Within these 2 groups the mosquitoes were randomly divided into groups of 6, placed in containers and held for 1, 6, 12, 24, 36, and 48 hours . A sugar solution was available *ad libitum*. After each time interval the designated mosquitoes were chilled to inactivation, placed in 1.5 ml tubes and frozen at -84°C until assayed (Figure 5).

2.7 Experiment 3. Detection in *Aedes togoi*

Aedes togoi have a wide latitudinal range and have adapted to lower temperatures than *Ae. aegypti*. This experiment examines the putative digestion rate and the duration of detection of MAb in *Ae. togoi* after selected time intervals.

Thirty-five individual *Aedes togoi* were fed MAb at a 1μg/ml concentration in blood (mean blood meal size = 3.0 μl, n = 5) and after feeding, the mosquitoes were chilled to inactivity, randomly divided into groups of 3 placed into containers and held

for 1, 6, 12, 24, 48 and 72 hours at 20°C and 70% relative humidity. After each time interval the designated mosquitoes were chilled to inactivation, placed in 1.5 ml tubes and frozen at -84°C until assayed (Figure 6).

2.8 Experiment 4. Air Drying, Alcohol Preservation and Faeces

These experiments tested air drying and alcohol fixation storage methods for blood fed mosquitoes as alternatives to freezing. Mosquitoes were assayed to determine if MAb could be detected after 3 weeks of storage. Mosquito faeces were also assayed to determine if MAb could be detected when digestion was complete.

Six individual *Ae. aegypti* that had been fed blood with 1 μg/ml MAb were held for 1 hour and then killed with ethyl acetate and allowed to air dry at room temperature in a perforated plastic container for 3 weeks. In another experiment, 6 fed mosquitoes were held for 1 hour and then preserved in 75% ethanol for 3 weeks. These mosquitoes were assayed to determine if the MAb was still detectable under these conditions.

After mosquitoes of both species had been fed blood with 1 μg/ml concentration of MAb and held for 24 hours, their faeces were removed from the containers by suspending them in 200 μl of PBS-T, and stored frozen at -84°C until assay.

2.9 Experiment 5. Blind Tests

Two blind assays were run to simulate a collection of feral mosquitoes, to determine if MAb-positive mosquitoes could be identified, and, if so, to estimate the rate of false positives.

Ae. aegypti were fed on 1 μg/ml concentration of MAb in blood and were held at 29°C for 1 hr or 24 hrs, and a control group of *Ae. aegypti* were fed on blood containing a 1:1000 dilution of the negative control isotype mouse IgG and held for 1 hr. All mosquitoes were frozen and then 12 mosquitoes from each group were numbered and randomized. The 36 mosquitoes were assayed blind in an ELISA along with controls and a set of reference dilutions. The mosquitoes were identified as one of two treatment groups or the control, based on their OD readings and comparison to their reference curves and the lower limit. The lower limit for positives was 0.05. The lower limit was based on preliminary experiments and the reference curve for the assay (Figure 7).

The second blind assay was run with the same groups with the exception that the number of mosquitoes in each group was unknown. A total of 30 mosquitoes were assayed. The lower limit for positives was 0.05. The lower limits were based on preliminary experiments and the reference curve for the assay (Figure 8).

I assess my ability to determine the presence or absence of MAb in the samples using a χ^2 test where the null hypothesis is a 50% success.

3 RESULTS

3.1 Experiment 1. Detection at Two Concentrations

When fed with an initial MAb concentration of 10μg/ml, the antibody could be detected in *Ae. aegypti* for 48 hours when held at 29°C and 60-70% relative humidity. Based on the OD reading there was relatively little change in the concentration of the MAb for the first 12 hours, a rapid decrease in concentration by 24 hours and the concentration was approaching the lower limit of detection at 48 hours. At the initial concentration of 1 μg/ml the MAb was detectable for 24 hours with a rapid drop in detectability between 12 and 24 hours (Figure 4). The polynomial regression lines are typical of general protein digestion curves for *Ae. aegypti* at this temperature (Hatfield 1988; Billingsley and Hecker 1991).

3.2 Experiment 2. Detection at Two Temperatures

MAb with an initial concentration of 1 μg/ml was detectable over 48 hours at 20°C with the most rapid decrease in OD between 24 and 48 hours. At 29°C the same initial concentration of MAb can be detected up to 24 hours and the most rapid decrease in OD is between 12 and 24 hours (Figure 5). The regression lines are polynomial. The 29°C line is typical of digestion in *Ae. aegypti* at this temperature. The 20°C line shows a different rate of digestion likely due to temperature effects on the enzyme secretion and enzyme kinetics.

3.3 Experiment 3. Detection in *Aedes togoi*

MAb was detectable for 72 hours when *Ae. togoi* were fed with an initial MAb concentration of 1μg/ml and held at 20°C and 70% relative humidity. Based on OD readings, the detectability of MAb decreased consistantly over 72 hours accelerating only slightly after 24 hours (Figure 6). To my knowledge there is no other research on protein digestion rates for *Ae. togoi*.

Figure 4. Comparison of putative digestion rates of the monoclonal antibody (MAb) in *Aedes aegypti* at 29°C, 1- 48 hours after feeding based on optical density (OD). Two concentrations (1μg/ml and 10μg/ml) of the MAb in blood were fed to mosquitoes. At an initial concentration of 1μg/ml MAb was detectable for 24 hours. At an initial concentration of 10μg/ml the MAb was detectable for 48 hours. The lower limit for positive OD = 0.05. Regression lines are polynomial. n = 3 per group. ●, ▲ = positive, negative control (10 μg/ml), O, Δ = positive, negative control (1 μg/ml).

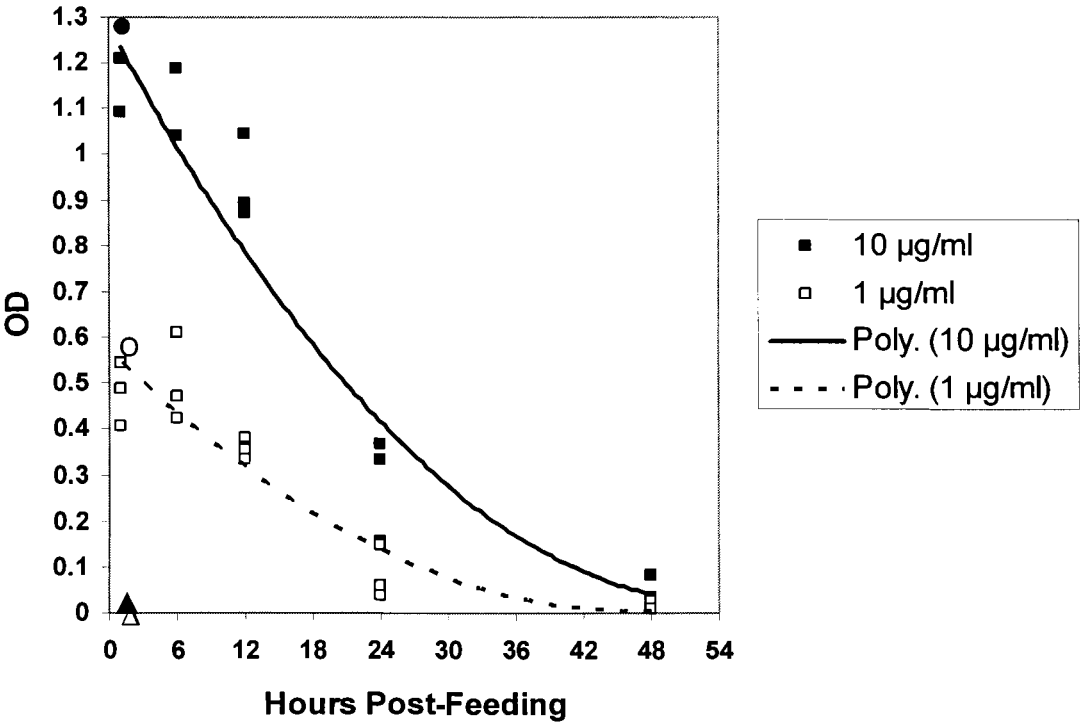
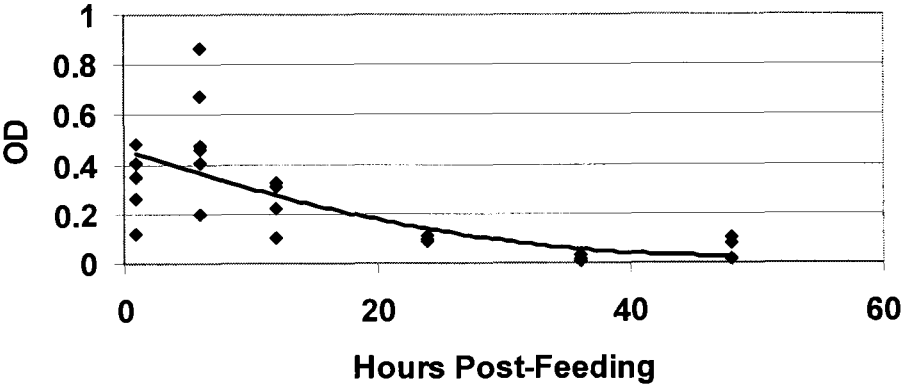

1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD
0
6
12
18
24
30
36
42
48
54
Hours Post-Feeding
10 µg/ml
1 µg/ml
Poly. (10 µg/ml)
Poly. (1 µg/ml)

Figure 5. Comparison of the putative digestion rates of the monoclonal antibody (MAb) in *Aedes aegypti* 1-48 hours after feeding on an initial MAb concentration of 1μg/ml in blood. Mosquitoes were held at two temperatures, 29°C or 20°C for 1, 6, 12, 24, 36 and 48 hours (n = 6 per group). MAb was detectable for 24 hours at 29°C and for 48 hours at 20°C. The lower limit for positives was considered to be OD = 0.05. Regression lines are polynomial.

29^{0}C


1
0.8
0.6
0.4
0.2
0
OD
0
20
40
60
Hours Post-Feeding

20^{0}C

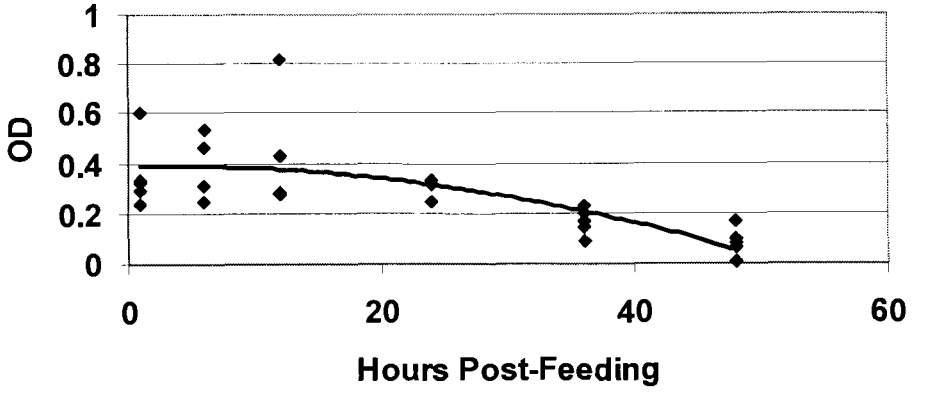
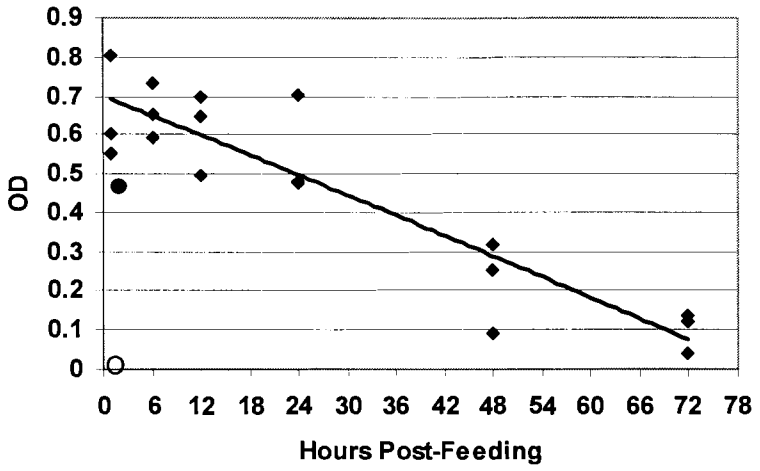

1
0.8
0.6
0.4
0.2
0
OD
0
20
40
60
Hours Post-Feeding

Figure 6. Putative digestion of monoclonal antibody (MAb) at 1μg/ml concentration in the blood-meal of *Aedes togoi* over 72 hours at 20°C based on optical density (OD). The lower limit for positives was considered to be OD = 0.05. ●, O = positive, negative controls.


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD
0 6 12 18 24 30 36 42 48 54 60 66 72 78
Hours Post-Feeding

3.4 Experiment 4. The Effect of Air Drying or Alcohol Fixation of Mosquitoes on MAb Detection

Ae. aegypti were fed blood with a 1 μg/ml concentration of MAb, killed after 1 hour with ethyl acetate and air dried. After 3 weeks at room temperature, the mean ELISA values were OD = 0.254 ± 0.106 at 405nm. Mosquitoes that were preserved in 75% ethanol were also positive for MAb after 3 weeks, with mean OD readings of 0.285 ± 0.130. In both treatments the readings were well above the lower limit of 0.05.

MAb were not detected in the faeces of either mosquito species. All readings were below the lower limit of detection (data not shown).

3.5 Experiment 5. Blind Tests

While there was a considerable variation within each group in the blind tests, it was possible to place nearly all of the mosquitoes in their group based on the preset limits. In the first blind test (Figure 7) the median value for the control was OD = 0.01, for the 24 hour group median value was OD = 0.09, and for the 1 hour group was OD = 0.22. There was 1 false negative (χ^2 test = 20.9, $p < .001$). In the second blind test (Figure 8) the median value for the control group was OD = 0.01, for the 24 hour group the median was OD = 0.09, and for the 1 hour group was OD = 0.27. There were 3 false negatives ($\chi 2$ test = 10.7, $p < .005$) The false negatives were likely caused by positive group mosquitoes that had failed to feed or had taken very small blood meals. There were no false positives.

Figure 7. Blind Test 1. Thirty-six *Aedes aegypti* fed on 1μg/ml monoclonal antibody (MAb) in blood. One group held for 1 hour, one group for 24 hours and there was a control group with no MAb in blood meal.Mosquitoes were held at 29°C. 12 mosquitoes in each group. Mosquitoes were assayed blind and identified to group from optical density (OD) readings. The lower limit for positives was considered to be OD = 0.05. There was 1 false negative and no false positives. n = 12 in each group. Horizontal bar indicates median values.

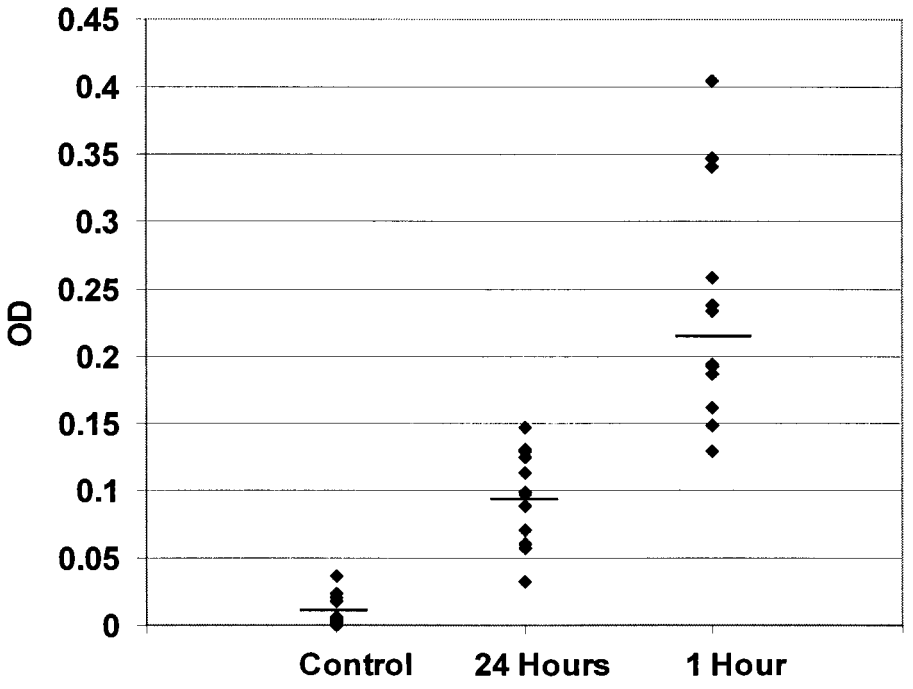
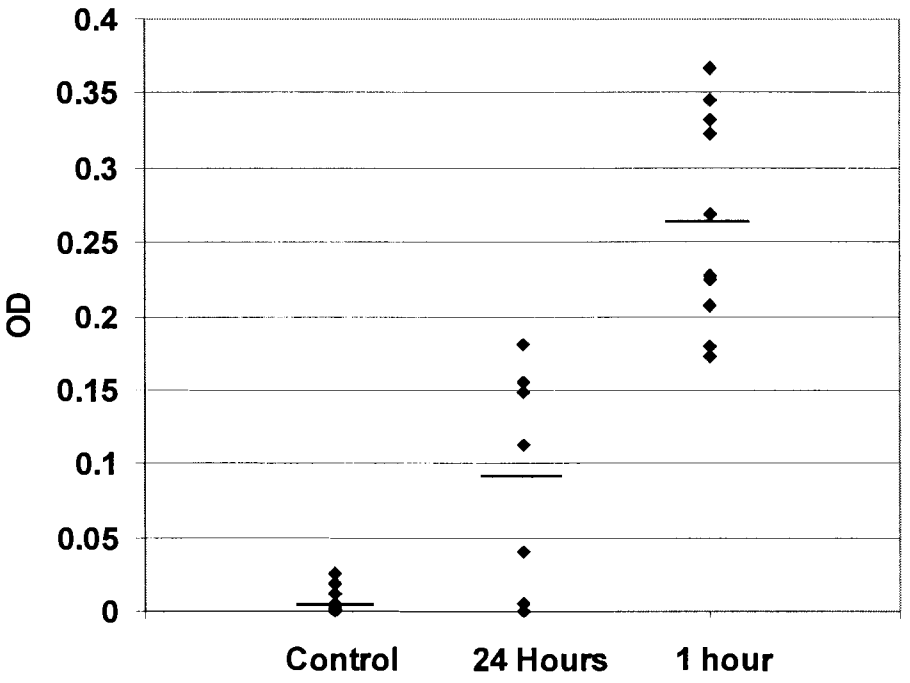

0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD
Control
24 Hours
1 Hour

Figure 8. Blind Test 2. Thirty *Aedes aegypti* fed on 1μg/ml monoclonal antibody (MAb) in blood. One group held for 1 hour, one group for 24 hours and a control group with no MAb in blood meal. Mosquitoes were held at 29°C. The number of mosquitoes in each group was unknown during the assay. Mosquitoes were assayed blind and identified to group from optical density (OD) readings. Lower limit for positives was considered to be OD = 0.05. There were 3 false negatives and no false positives. Median value lines for each group are shown. Control n = 13, 24 Hour n = 7, 1 Hour n = 10. Horizontal bar indicates median values.


0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD
Control
24 Hours
1 hour

4 DISCUSSION

4.1 General Discussion

These results support the hypothesis that antibodies in vertebrate blood can be detected in mosquito blood meals using an ELISA assay. In this study specific IgG could be detected for 24 to 72 hours after feeding. The limits to detection depended on temperature, and on the initial concentration of MAb in the mosquito blood meal. These initial concentrations of MAb (10 μg/ml and 1μg/ml), are within the normal range of specific IgG (1 pg/ml-10mg/ml) in the blood of seropositive mammals (Harlow and Lane 1999).

Ae. aegypti was chosen for this study because it is a well-established laboratory species and has been used in a number of related studies of antibodies in mosquitoes. It is a tropical mosquito with a wide geographical range and it is reared at high temperatures (26-29°C). At 29°C the blood meal was rapidly consumed and MAb was detectable only for 24 hours at the low MAb concentration (1μg/ml) and 48 hours at the higher concentration (10μg/ml). When post-feeding *Ae. aegypti* were held at 20°C their physical activity and presumably their digestion rate were reduced, and MAb was detectable for 48 hours at an initial concentration of 1 μg/ml.

Ae. togoi has a much broader latitude range, it is found in the tropics and on the coastlines of British Columbia and Russia, north of the Sea of Japan (Belton 1983). This mosquito was chosen as it is found on the sea coasts and may be of interest to researchers

working with disease in humans, marine mammals and birds. This mosquito is much more adaptable than *Ae. aegypti* to low temperature. *Ae. togoi* was held at 20°C post-feeding and the MAb could be detected in the assay for 72 hours after feeding on an initial concentration of 1 μg/ml.

The indirect ELISA was chosen because it is a simple system that could be adapted for field use. A monoclonal antibody was used and its value in this study is uncertain. Using a monoclonal antibody should limit non-specific binding but it has only one type of binding site on the antigen as it is specific to a single epitope or antigenic determinant. Most antigens have a variety of epitopes (Alberts et al. 1994) and polyclonal antibodies that would normally be found in blood, bind with a number of epitopes on an antigen and may give a stronger signal at the same concentration depending on the nature of the antigen.

The blind tests examined the validity of the preset lower limits that allowed for identification of positives with few false negatives and no false positives, these tests also served as a simple simulation of testing wild caught mosquitoes. The lower limit for positives of OD = 0.05 was based on preliminary experiments and this limit also represents more than 3 times the highest background level of the assays in this study. Kemeny (1991) recommended lower limits of 1.5 times the background. While there were no false positives, the negative controls were as high as OD = 0.04, and the lower limit of 0.05 may not be adequate. A more appropriate lower limit for this assay would be OD = 0.1. χ^2 tests indicated that the results of the blind tests were highly significant.

In field diagnostic studies there will always be a chance of having false negatives if mosquitoes with very small blood meals are used or if too much time has passed since the blood meal was taken. Selection of well-fed mosquitoes would help to reduce this problem. False positives are a more serious problem as they could lead to a false diagnosis. The use of standardized positive and negative controls and a pre-determined lower limit can reduce the chance of false positives occurring.

The detector antibody with the conjugated enzyme used to detect bound MAb is a goat anti-mouse IgG1. This antibody has a whole molecule specificity to IgG1 which may improve detection as it can detect fragments of IgG as long as they still retain the Fab and $F(ab')_2$ binding sites. If the detection antibody were specific to the Fc fragment, the cleaved antibody might not be detected as the Fab and $F(ab')_2$ fragments would be missed and the Fc fragment which lacks binding sites would not bind to the antigen on the plate. It is possible that IgG in the blood meal is cleaved by trypsins in the early stage of digestion. Preliminary assays in this study using an Fc specific detector antibody for MAb worked as well as the whole molecule antibody and this suggests that intact MAb is present in the blood meals (Vaughan et al. 1998).

IgG was chosen because it is the most abundant Ig class, and it is believed to be one of the most persistent proteins in mosquito blood meals (Irby and Apperson 1989). IgG has been successfully detected in blood-feeding arthropods in other studies (Minoura et al. 1985; Ben-Yakir et al. 1987; Hatfield 1988; Vaughn et al. 1998). IgG is a normal part of the protein component of mosquito blood meals and the putative digestion rate of MAb in this study is typical of other protein digestion studies (Houk and Hardy 1982; Irby and Apperson 1989; Billingsley and Hecker 1991). For example, Irby and Apperson

(1989) reported that IgG persisted for 36-48 hours after feeding in *Ae. aegypti* when mosquitoes are held at 26°C.

The apparent absence of MAb in the mosquito faeces suggests that MAb is completely digested in the gut or passed into the haemolymph, although trypsin activity in the faeces (Briegel 1975) may have destroyed any remaining MAb. This study was done using whole mosquito homogenates as this would be most practical in the field and because IgG may also be found in the mosquito haemolymph (Hatfield 1988).

It should be possible to use a single blood fed mosquito for both host antibody analysis and host identification. Service et al. (1986) could identify hosts from as little as 0.02 µl of blood. In this study MAb was detectable in whole mosquito homogenates that had been diluted to 200 µl, so multiple assays with a single mosquito are possible. Pooling a number of blood-fed mosquitoes may improve detection and increase the post-feeding time period over which specific antibodies can be detected but pooling mosquitoes may cause problems by mixing blood from different host species.

When multiple blood meals in a mosquito are from individuals of the same species or closely related species, it may be difficult to distinguish between host antibodies. If the mixed blood meal comes from more distinct taxonomic groups, the detector antibody can distinguish the source of the specific antibodies. For example, if a mixed blood meal contains deer and rabbit blood and the detector antibody was an anti-rabbit IgG antibody, it is unlikely to react with deer IgG. The detector antibody would be chosen for the species or general taxonomic group being monitored. Molecular techniques such as PCR could also provide more specific host identification (Gokool et al

1993; Boakye et al. 1999; Ngo and Kramer 2003). The amplification of cytochrome B gene sequences by PCR and their sequencing showed sufficient interspecific variation to distinguish between mammalian host samples in mosquito blood meals (Boakye et al. 1999).

Edman and Schmid (1970) demonstrated that mosquitoes with blood meals could be stored for periods up to 4 years and that the host blood could still be identified using precipitin-tests. Bangs et al. (2002) were able to quantify *Plasmodium* circumsporozoite proteins in the bodies of wild-caught mosquitoes that had been desiccated and transported in tubes with silica gel. In the present study, MAb could be detected in mosquitoes that had fed on MAb blood and were killed with ethyl acetate and air dried for 3 weeks. This method, as well as alcohol preservation could be useful for the collection and transporting of mosquitoes for studying antibodies in areas where refrigeration is not available. Dried mosquitoes could be useful for examining the identity of the host, detecting the proteins of pathogens, and examining host antibodies to pathogens that are ingested by the mosquito.

Many improvements could be made to this system for specific uses or for more sensitive assays. The semi-quantitative detection of IgG may not distinguish between animals with active infections and animals that are seropositive without infections. Electrophoretic methods such as immunoblots can distinguish between IgG and IgM. If IgM was detectable in arthropod blood meals, and its concentration in the blood in relation to IgG were determined, it may provide a better indication of acute infection.

One other possibility that could provide supplementary information about the host is assaying for haptoglobin levels with ELISA. Increased haptoglobin levels are a non-specific indicator of inflammation that is likely to occur with infection (Zenteno-Savin et al. 1997). A surveillance system could monitor for haptoglobin levels or other general indicators of disease and if increased levels were observed then more specific monitoring and diagnostic methods could follow.

This method of detecting host antibodies to pathogens in mosquitoes using ELISA may be most useful for detecting infections where the pathogen is sequestered in specific tissues of the vertebrate host, and the pathogen or its antigens are not continuously circulating in the blood, but antibodies specific to the pathogen are present in the blood at detectable levels. Examples of this include parasitic diseases caused by cestodes or nematodes encysted in deep tissue. For example, Echinococcosis, caused by the cestode *Echinococcus* spp. forms cysts in the liver or lungs and can be transmitted to domestic animals from wild canids (Cheng 1986). Cysticercosis is caused by the larval cestode *Taenia* spp. which encysts in the central nervous system including the brain. Trichinosis caused by nematodes of the genus *Trichinella* encyst in muscle tissues particularly the diaphragm and can damage heart muscle. Sylvatic trichinosis occurs in bears, badgers, foxes and walruses, urban trichinosis can transmit between rats, pigs and humans (Schmidt and Roberts 1989). No eggs are shed by the hosts of the cestodes and larval stages of *Trichinella* are rarely found in host faeces (Schmidt and Roberts 1989).

Diagnosis is dependant on detection of antibodies in the blood or by biopsy of affected tissue. Commercial antibody detection systems (ELISA) are available for these infections (Cypress Diagnostics, Belgium) and could potentially be used with blood fed

mosquitoes. With development, these techniques could become a useful tool for disease surveillance and for early warning of epizootics.

4.2 Future Directions

There are a growing number of detection techniques that could be applied to monitoring disease using mosquito blood meals. Dipsticks are a one step ELISA and can provide a useful field tool. Dipsticks are plastic strips made with nitrocellulose or other high capacity matrix (Kemeny 1991). These have been used for detecting antigens specific to *Plasmodium* sporozoite antigens in mosquitoes and can provide results in 15 minutes after exposure to homogenized mosquito suspensions (Bangs et al. 2002). Dipsticks can also be used to detect antibodies. Antibody detecting dipsticks and reader technologies are commercially available to test for a number of diseases (Response Biomedical Corp. Burnaby B.C.) and these technologies could be adapted for monitoring many diseases using blood fed mosquito homogenates.

Another type of ELISA that could be useful is an antibody-capture ELISA where an Fc binding protein such as Protein A, Protein G or Protein L is coated on the plate and binds to sample antibodies by the Fc region (Harlow and Lane 1999). While any antibodies with an Fc region may be bound to the Fc binding protein, bound antibodies that are specific to an antigen can be identified by the addition of enzyme-conjugated antigen. This type of assay is not dependent on a specific detector antibody and could be useful in monitoring all the species in an area for a specific pathogen. By retaining a portion of the samples, blood from positive results could be used to identify the host.

As detection technology becomes increasingly sensitive, mosquito blood meals could possibly be put to other uses such as monitoring pesticides, heavy metals and other environmental toxins in animals or they could be used for monitoring other animal blood components that can be used to determine the nutritional health of animals or to look for signs of non-infectious disease.

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