SHORT COMMUNICATION

Comparison between Precipitin and ELISA Tests in the Bloodmeal Detection of *Aedes aegypti* (Linnaeus) and *Aedes fluviatilis* (Lutz) Mosquitoes Experimentally Fed on Feline, Canine and Human Hosts

Luciana AM Gomes/+, Rosemere Duarte*, Dinair C Lima, Beatriz Sanson Diniz*, Maria Lucia Serrão***, Norma Labarthe**

Faculdade de Veterinária, Centro de Ciências Médicas, Universidade Federal Fluminense, Rua Itaocara 88, 24240-100 Niterói, RJ, Brasil *Laboratório de Imunodiagnóstico, Departamento de Ciências Biológicas, Escola Nacional de Saúde Pública-Fiocruz, Rio de Janeiro, RJ, Brasil **Laboratório de Transmissores de Hematozoários, Departamento de Entomologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil ***Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil

The identification of arthropod bloodmeals is important in many epidemiological studies, as, the understanding of the life cycle of vectors and the patogens they transmit, as well as helping to define arthropods' control strategies. The precipitin test has been used for decades, but ELISA is slowly becoming more popular. To compare the two tests for sensitivity, specificity and accuracy to detect small insect bloodmeals, Aedes aegypti *or* Ae. fluviatilis *mosquitoes were fed either on feline, canine or human hosts. Mosquitoes were frozen at 6, 12, 24, 48 or 72 h after feeding. Precipitin test showed better specificity and accuracy and ELISA test showed higher sensitivity. Better results with both tests were achieved when mosquitoes were frozen within 48 h from feeding.*

Key words: bloodmeal identification - mosquito - ELISA - canine blood - feline blood - human blood

The determination of mosquitos' bloodmeal helps to understand pathogen life cycles, their potential hosts and to identify control strategies (Boreham 1975, Tempelis 1975, Ngumbi et al. 1992). Precipitin tests have been used to detect insects' bloodmeals for many decades (Bull & King 1923) and since the 80's ELISA has been introduced as a better alternative (Burkot et al. 1981). Each test has its advantages and restrictions and should be choosen for better accuracy. The present study was conducted, in order to find a comparison in sensitivity, specificity or accuracy between the two tests for bloodmeal identification in mosquitoes.

The mosquitoes used in the present study were raised in laboratory and their species were either *Aedes aegypti* (Linnaeus) or *Ae. fluviatilis* (Lutz). Immunized rabbit serum with total proteins of feline, canine and human serum (Gill 1984) were used in both tests after being titered and absorbed to higher specificity (Weitz 1952, Duarte 1997). Ninety-five young mosquito females were fed once, for 60 min, on dogs, cats or human volunteer (total of 285 mosquitoes). After feeding, mosquitoes were kept in groups of 19 and each group was frozen at 6, 12, 24, 48 or 72 h and kept at -20°C until processing. Digestive contents were taken and diluted at 1:10 in PBS 0.01 M – pH 7.2 (Beier et al. 1988) to the precipitin test. Before the dilution, the color of each sample was observed subjectively, recorded, and unfed mosquitoes were sorted out. Negative controls were obtained using the above technique with unfed mosquitoes. Antiserum reached titers 1:30,000 (anti-feline and anti-human) and 1:16,000 (anti-canine). Results were recorded double-blind. ELISA tests were run with the same samples and antisera used for precipitin, although antisera were diluted at 1:5,000. Samples were diluted, in double, at 1:50 in carbonate bicarbonate buffer (pH 9.6) and applied to 96 well polystyrene microplate (Alfesa, SP, Brasil). After incubation, plates were washed once in PBS 0,01 M added with tween 20 diluted 0.05% and dried. Diluted antiserum was than added to the wells and

+Corresponding author. Fax: +55-21-714.9302. E-mail cmgomes@cruiser.com.br
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the microplate incubated at 37°C for 45 min. After another wash, the conjugate was added, and after another incubation and wash, the substrate was applied. The plates were analyzed in microplate reader using 492 nm operational filter and 600 nm reference filter (Burkot et al. 1981). Cut off was established as the mean of negative control plus two standard deviations. Positive samples were those reading 10% over the cut off. Sensitivity, specificity and accuracy were stablished according to Rouquayrol (1994).

Approximately 80% of the colorless samples did not react, suggesting that those mosquitoes did not blood-feed enough to show any reaction and were therefore discarded. Both tests showed better sensitivity when mosquitoes were frozen less than 24 h after the bloodmeal. Precipitin test specificity was high when using anti-human or anti-canine serum with mosquito bloodmeals less than 24 h before freezing. However, the anti-feline serum was the least specific. The anti-feline antiserum system did not fall below 79% within 12 h (Table I). ELISA test results were always lower in specificity (Table I), and showed better sensitivity when compared to precipitin (Table II), and when accuracy is taken into consideration, precipitin showed to be a stronger test (Table III).

In an overall analysis, precipitin showed slightly better results, especially when specificity was the main requirement or when accuracy was taken into consideration. Perhaps in situations where sensitivity is to be highlighted, ELISA may be the choice. One must keep in mind that specificity and sensitivity were better when samples came from mosquitoes fed less than 48 h before digestion process was stoped by freezing, as stated (Bukort et al. 1981, Ngumbi et al. 1992, Savage et al. 1993). The system to detect canine blood in mosquitoes' gut was less sensitive than the system to detect feline blood, which can be explained by the difference in the antiserum titers. In the present paper, the precipitin test showed to be more sensitive and specific than ELISA to detect mosquitoes' bloodmeal origin, besides being easy to perform and being a low cost technique.

TABLE I

Precipitin and ELISA tests specificity in identifying bloodmeal of laboratory raised *Aedes aegypti* or *Ae. fluviatilis* after feeding on a known host

		Time after feeding (%)				
Antisera		6 h	12 h	24 h	48 h	72 h
Feline	Precipitin	96	79	88	77	100
	ELISA	88	83	73	82	56
Canine	Precipitin	100	100	97	89	100
	ELISA	82	83	90	93	73
Human	Precipitin	100	100	100	100	100
	ELISA	94	96	78	64	100

TABLE II

Precipitin and ELISA tests sensitivity in identifying bloodmeal of laboratory raised *Aedes aegypti* or *Ae. fluviatilis* after feeding on a known host

		Time after feeding (%)				
Antisera		6 h	12 h	24 h	48 h	72 h
Feline	Precipitin	100	88	81	7	-
	ELISA	100	54	56	7	-
Canine	Precipitin	27	54	73	-	-
	ELISA	7	62	100	50	-
Human	Precipitin	83	64	27	14	38
	ELISA	-	64	73	-	25

- : without reaction

TABLE III

Precipitin and ELISA tests accuracy in identifying bloodmeal of laboratory raised *Aedes aegypti* or *Ae. fluviatilis* after a feeding on a known host

		Time after feeding (%)				
Antisera		6 h	12 h	24 h	48 h	72 h
Feline	Precipitin	98	83	86	50	75
	ELISA	93	73	67	53	42
Canine	Precipitin	76	85	90	69	92
	ELISA	56	76	93	83	67
Human	Precipitin	96	90	74	67	58
	ELISA	72	86	76	39	50

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