Development and Characterization of an Anti-Bat Antisera and its Implementation in Screening for Rabies Antibody in Bats via ELISA and IFA

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INTRODUCTION

Bats have long been associated with the transmission of a number of zoonotic agents, including rabies.

•Since 1990, bat rabies variants have accounted for 24 (75%) of the 32 cases of human rabies in the U.S.

•In 1996, the first case of human rabies since 1972 in Chile was caused by a bat rabies variant.

•Recent epizootics in South America involving human and canine cases have also been linked to bat variants.

Comprehensive surveys of wild bat populations to characterize the seroprevalence rate of bat populations could prove helpful in implementing control programs designed to reduce the number of human and veterinary case resulting from rabies bat variants. There are two problems associated with techniques currently used to measure antibodies in bats to rabies virus:

•Use of live rabies virus, which is a significant biohazard

•Requirement for a large amount of serum relative to what can be obtained from bats

OBJECTIVES:

•Develop an antiserum to detect immunoglobulins (Ig) from the Big brown bat (*Eptesicus fuscus*)

•Characterize the anti-bat Ig serum for cross-reactivity with other species, including other bat species.

Apply the anti-bat Ig reagent to detection of anti-rabies antibodies using enzyme-linked immunosorbant (ELISA) and indirect immunofluorescent (IFA) assays.

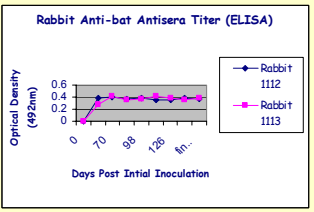
PREPARATION OF ANTISERA

• Rabbit anti-bat antiserum was developed by immunizing rabbits with purified *Eptesicus fuscus* IgG using a commercial affinity chromatography kit (*Amersham Biosciences).*

• Rabbits were initially inoculated sc 200 μg *E. fuscus* IgG + Freunds complete adjuvant and administered 4 IM boosters every 2 weeks with 200μg + Freunds incomplete adjuvant.

• Rabbits were bled every two weeks to determine antibody levels

• Immunoglobulins were purified from immune rabbit sera and labeled with horseradish peroxidase (HRP).

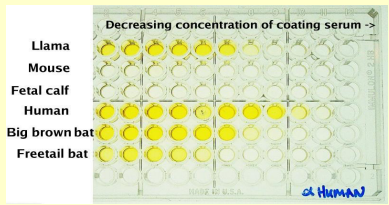


Immunoblot showing crossreactive against non-immunoglobulin proteins for commercial antisera and anti-bat Ig antiserum

CHARACTERIZATION AND SPECIFICITY OF OUR RABBIT ANTI-BAT IgG ANTISERA

•Coated Immulon-2 Plate with different animal sera at 1:100, followed by subsequent half-log serum dilutions

•Tested our rabbit-anti bat IgG-HRP antiserum and a commercial anti-human-HRP (KPL laboratories, Inc) for species specificity



Comparable cross-reactivity results were obtained from our antisera when compared to a commercially available antibody from KPL Laboratories.

PRELIMINARY ELISA AND IFA FINDINGS

ELISA

•Immulon-2 plates coated with rabies mouse brain antigen (CDC, Fort Collins, CO) at 1:500 in carbonate-bicarbonate coating buffer

•Blocked using 5% nonfat dry milk + NaN_3

•Known seropositive and seronegative samples diluted in blocking buffer tested at serial 2-fold dilutions with a starting dilution of 1:20

•HRP-labeled antisera was incubated for one hour then washed

•Plates were developed with TMB Peroxidase Substrate (KPL Laboratories) for 15 minutes, stopped with 1M phosphoric acid and read at 492 nm using an automatic plate reader.

Optical density (492 nm) readings from rabies ELISA

	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
kelsey	0.273	0.252	0.221	0.178	0.136	0.106	0.080	0.083
melissa	0.230	0.200	0.154	0.116	0.089	0.072	0.062	0.055
elisabeth	0.136	0.108	0.085	0.074	0.061	0.061	0.054	0.046
bat 1028			0.198	0.179	0.163	0.168	0.122	0.125
bat 1015			0.103	0.096	0.098	0.078	0.065	0.064

IFA

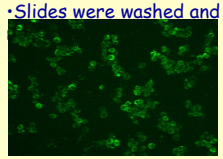
•Slides were prepared by acetone-fixing baby hamster kidney cells roughly 50% infected with rabies Challenge Virus Strain (CVS)

•Serial two-fold dilutions of bat serum starting at a 1:10 dilution in PBS were made and allowed to incubate overnight at 4°C overnight

•Purified rabbit anti-bat antisera was applied at a 1:50 dilution and incubated for 30 minutes at 37 °C

•Slides were washed in PBS and a commercial Fluorescein Isothiocyanate (FITC) goat anti-rabbit (Jackson ImmunoResearch) was applied at a 1:50 dilution and incubated for 30 minutes at 37°C

•Slides were washed and observed by fluorescent



Indirect immunofluorescence with CVS-infected BHK cells reacted with bat anti-rabies serum and rabbit anti-bat Ig.

CONCLUSIONS

•An antiserum was successfully developed and purified to detect immunoglobulins from the Big Brown Bat (*E. fuscus)* The antiserum showed some cross reactivity with llama serum and cross reactivity with serum from the Mexican Freetail bat (*Tadarida brasiliensis).* When compared to a commercial HRP-conjugate, our HRP-labeled antisera showed comparable specificity when cross-reacted with sera from several mammalian sera.

•Preliminary results from our ELISA and IFA are encouraging and show promise for implementation in the development of a rapid screening tests which, once optimized, could be use on a wide scale basis to test for seroprevalance of rabies in wild bat populations.

•The screening tests being developed confer significant advantages over the current techniques used to screen bat populations for rabies specific antibody. Both negate the propogation of live virus and both require small amounts of sera for testing.