RABIES: A GLOBAL THREAT

"Taking a Lead on Education and Scientific Initiatives "

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Masters of Public Health Defense
Emphasis: Infectious Disease and Zoonoses

April 17th 2014

"For centuries man has felt terror after bites by rabid dogs".

Dr. George M. Baer (1/12/36 – 6/2/09)

RABIES: A GLOBAL THREAT

- ✓ **INTRODUCTION**
  - **- HISTORY**
  - **- VIRUS & PATHOGENSIS**
  - **- DIAGNOSIS**
  - **- VACCINE & PROPHYLAXIS**
  - **- HUMAN CASE**
  - **- GLOBAL BURDEN**
- ✓ **FIELD EXPERIENCE**
- ✓ **CAPSTONE EXPERIENCE**
- ✓ **RECOMMENDATIONS**

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Masters of Public Health Defense
April 17th 2014

Kansas State University

History of Rabies

- Ancient Literature: Traced back more than 4000 years…
  - **2300 BC**: Eshnunna – 1st Documentation. If a dog is mad, authorities notify owner, dog not kept in, dog bites man, results in man's death then owner pays 2/3 a mine of silver. (Baer, 2007)
  - **500 BC**: 1st Documentation Case of Canine Rabies by Greek Philosopher Democritus. (A Short History of Rabies, 2013)
  - **800-700 BC**: "*The Iliad"* written by Homer(A Short History of Rabies, 2013)
  - **400 BC**: Natural History of Animals, Author Aristotle (Hernandez, 2009)
  - **~400 BC**: 2 Gods Identified – Arisaeus, prevents rabies & Artemis, cure rabies (A History of Rabies, 2013).

History of Rabies

- Ancient Literature: Traced back more than 4000 years…
  - **001-100 AD**: Rabies Spread Across Roman Empire
  - **1271**: 1st Outbreak Recorded – 30 human deaths, Germany from wolves.
  - **1400s**: Spreads Across Spain
  - **1700s**: Spreads Across Europe During 18th Century
  - **1703**: 1st Noted in Americas by a Priest in Mexico
  - **1734-5:** Canine Rabies Appears in England
  - **1800:** European Villagers Dying from Contact with Mad Wolves, Foxes and Canines.
  - **1835:** Appears in Chile and Kills Many
  - **1953 AD:** 1st Case Bat Rabies USA by CDC

Reference: A History of Rabies, 2013

History of Rabies

Myths: Cause, Transmission and 'Cures' - play instrumental role in society.

Myths Include:

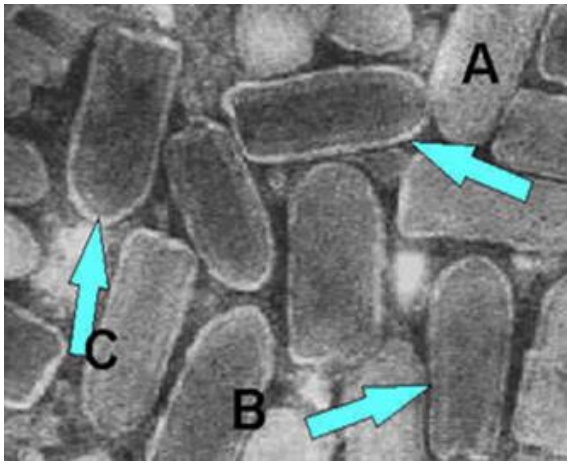
- Physically sucking wounds to remove poisons
- Placing the rabid animal hair over the human wound = a cure
- Infected children consuming raw heart & liver of infected K-9 = cure
- Placing the heart or tongue of infected animal in shoe would prevent rabies
- St. Hubert's Key
- Witches cause rabies by spells and evil spirits
- Consumption of maggots from rabid animal = a remedy

The word rabies stems from the Latin word rabere which is defined as rave or rage. It is believed that the word rabere is rooted to the Sanskrit word rabhas which is defined as doing violence

("A short history of rabies","2013).

Virus Introduction & Pathogenesis

- Rabies is an acute (neurotropic virus)
- Zoonotic – primarily spread through an infected bite
- Routes of transmission: infected bite, aerosol transmission, mucosal contact (mouth, nose, eyes), licking of broken skin, transplantations of organs and corneas, penetration of skin from scratch with bleeding
- Non-bite transmission = rare in humans, but do and can occur
- Excluding transplantations – human to human cases never documented
- Best described as enveloped, rod or bullet shape
- Rhabdovirdae family → Lyssavirus genus
- Greek language: rhabdos = ‘rod’, lyssa = ‘rage’
- Two Forms:
  - Paralytic (“Dumb”)
  - Hyperactivity: Aggressive / Furious



Negatively Stained Rabies (Rhabdovirus) through electron microscope (CDC, 2014)

Virus Introduction & Pathogenesis

- Size: 180 x 75 nanometers
- Encodes 5 proteins
  - (N) – Nucleoprotein

    Encapsidates (encloses) RNA
  - (P) – Phosphoprotein

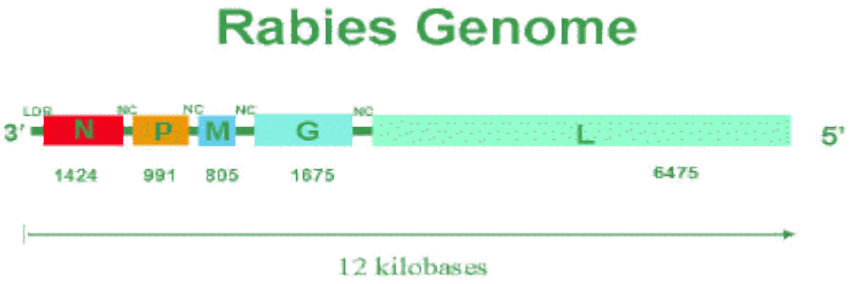
    Aid in replication process
  - (L) – Polymerase

    Aid in replication process
  - (G) – Glycoprotein

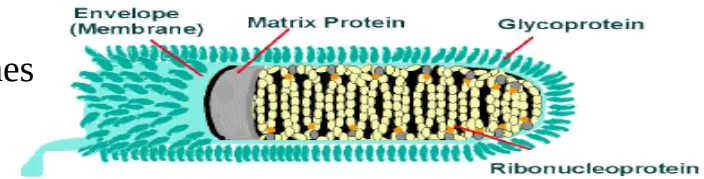
    On surface, spiked, attaches to host membranes
  - (M) – Matrix Protein

    Key in assembly and egress

    Important role in giving the 'bullet' shape



The order and the size of the proteins is imperative as this makes up the virus. (Centers for Disease Control and Prevention, 2014).



Centers for Disease Control and Prevention, 2014

Life cycle of rabies infection = 3 phases: 1) Attachment of virion to susceptible membrane, 2) Replication of virion , most difficult, and 3) final replication and budding.
(Wunner, 2007)

Virus Introduction & Pathogenesis

→Recall: Highly neurotropic
→Virus enters subject – replicates at or near entry site
↓Enters peripheral nerves
↓Travels to CNS (replication continues)
↓Travel out to organs, including salivary glands
Salivary glands = primary exit portal to be passed on to hosts

⇨ Wound Management!!

<u>Clinical Symptoms:</u>

* Rabies = 99.9% Mortality Rate (GARC, 2014)

- Headache, fever, sore throat
- Increased sensitivity to noise and light
-Hallucinations, ↑libido, nightmares
-Aerophobia, hydrophobia, depression
- Encephalitis

Diagnosis

Michael Peres, 2014

DFA – Direct Fluorescent Antibody (Gold Standard)

- Post-Mortem
- Brain Stem – Cerebellum (**Must Test Negative**), Hippocampus (CDC, 2014)
- Utilizes Antibodies to Attach to the Rabies Virus (Antigen)
- Brain Tissue Required as Virus Resides in Nervous Tissue
  (Not Blood as Other Viruses)

*** Mammals: Excluding Euthanasia = 10 day Quarantine**

Anti-mortem Diagnosis - Human: Detect Antigen, Nucleic Acids or Virus Isolation

- Saliva
- Corneal Impressions
- Eye Wash Fluid
- CSF
- Tears
- Nuchal Biopsies

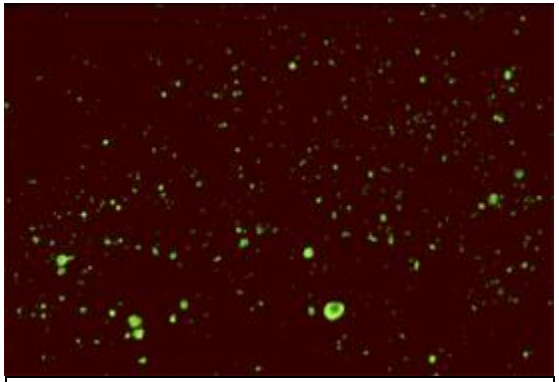


Figure 1.7: DFA test viewed through a fluorescent microscope that is positive for rabies, (Centers for Disease Control and Prevention, 2014).

Vaccine, Prophylaxis and Immunoglobulin

Risk Category	Nature of Risk	Typical Population	Pre-exposure Recommendations
Continuous	Virus present continuously, often in high concentrations. Specific exposures likely to go unrecognized. Bite, nonbite, or aerosol exposure.	Rabies research laboratory workers; rabies biologics production workers.	Primary course. Serologic testing every 6 months; booster vaccination if antibody titer is below acceptable level.
Frequent	Exposure usually episodic, with source recognized, but exposure also might be unrecognized. Bite, nonbite, or aerosol exposure.	Rabies diagnostic lab workers, spelunkers, veterinarians and staff, and animal-control and wildlife workers in rabies-enzootic areas. All persons who frequently handle bats.	Primary course. Serologic testing every 2 years; booster vaccination if antibody titer is below acceptable level.
Infrequent	Exposure nearly always episodic with source recognized. Bite or nonbite exposure.	Veterinarians and terrestrial animal-control workers in areas where rabies is uncommon to rare. Veterinary students. Travelers visiting areas where rabies is enzootic and immediate access to appropriate medical care including biologics is limited.	Primary course. No serologic testing or booster vaccination.
Rare (population at large)	Exposure always episodic with source recognized. Bite or nonbite exposure.	U.S. population at large, including persons in rabies-epizootic areas.	No vaccination necessary.

Pre-Exposure Prophylaxis

Vaccine, Prophylaxis and Immunoglobulin

Type of vaccination	Route	Regimen
Primary	Intramuscular	Human diploid cell vaccine (HDCV) or purified chick embryo cell vaccine (PCECV); 1.0 mL (deltoid area), one each on days 0,* 3, 7, and 14
Booster†	Intramuscular	HDCV or PCECV; 1.0 mL (deltoid area), day 0 and 3

Post-Exposure Prophylaxis

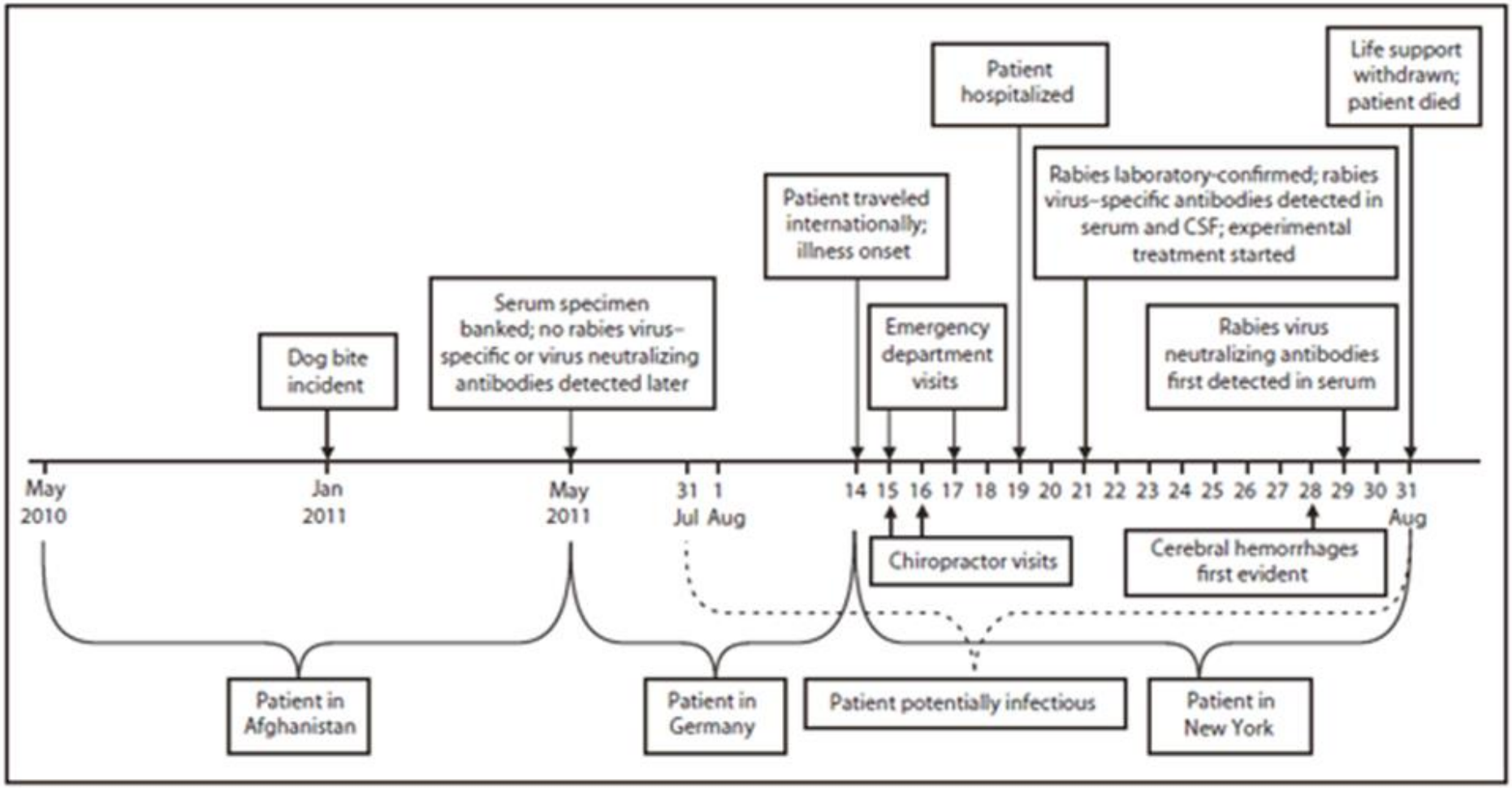
RIG & ORVs

"…rabies has one of the highest case-fatality ratios of any infectious disease"

(Compendium of Animal Rabies Prevention and Control, 2011)

Human Rabies Case - Timeline

Private First Class Kevin Shumaker: 24 years, Bite to R hand from feral/community K-9 while deployed in Afghanistan in January 2011. August 14th = 1st Clinical Symptoms.



Human Rabies Case - Timeline

Pfc. Kevin Shumaker. Photo taken October 28, 2010 (Mail Online, 2012)

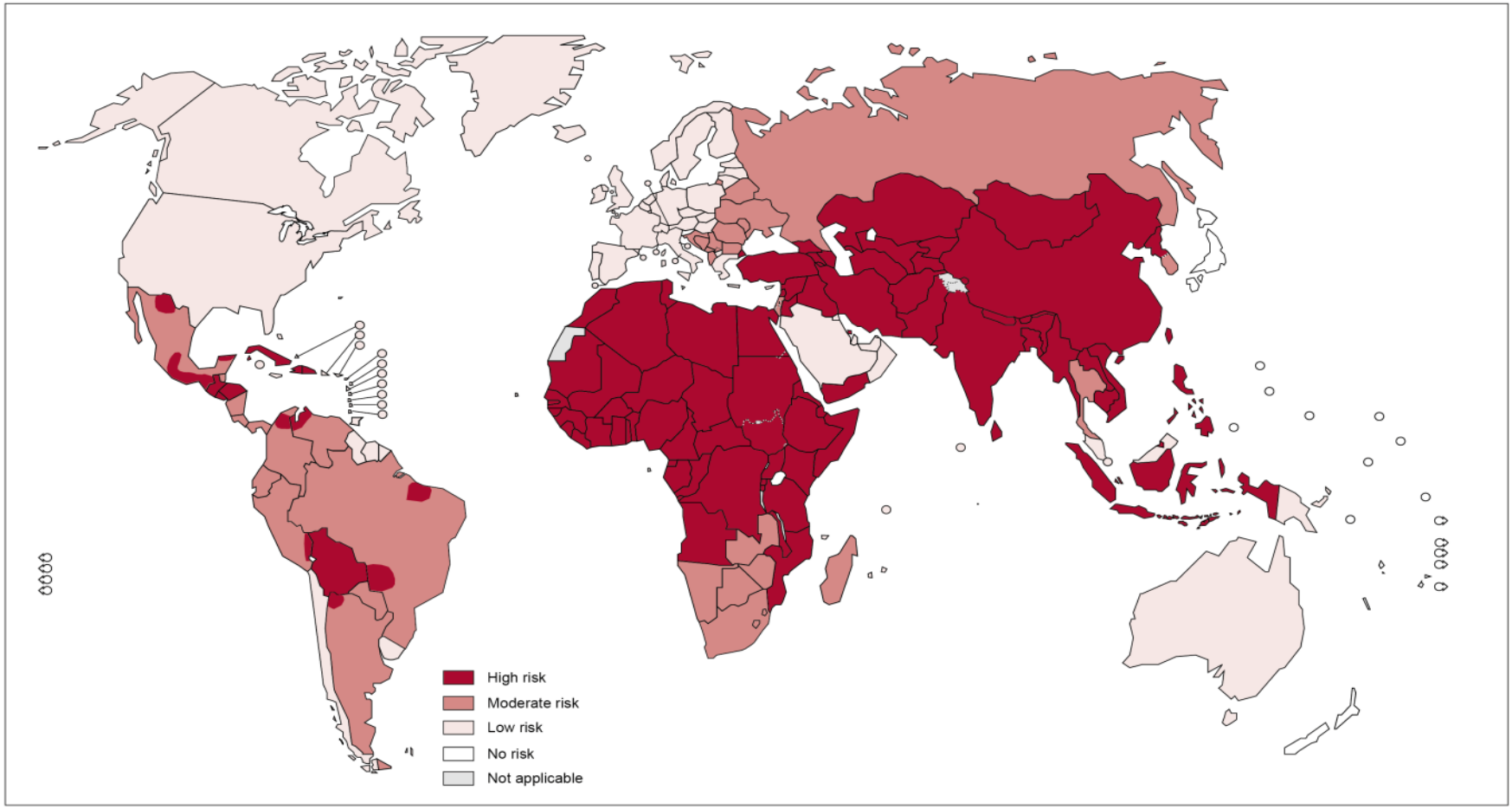
Global Burden of Rabies

- Rabies is found on 6 of the 7 world continents (OIE, 2014)
- Canines = main global source (GARC, 2013)
- PRP Study Reflects ~61,000 Human Deaths Annually (WHO, 2012)
- 95% Africa / Asia - - India = Highest Reported Incidences (GARC, 2013)
- 60% → Children 15 yrs. and younger (GARC, 2013)
- 84% Deaths Occur is Rural Areas (Poorest & Most Remote) (GARC, 201
- 3.3 Million People Live with Rabies Risk Daily (GARC, 2014)

Globally, dogs are the main source of rabies as over 95% of human deaths result from infected dogs. ***(Dr. Deborah Briggs, 2013)***

Global Burden of Rabies

Distribution of risk levels for humans contacting rabies, worldwide, 2011



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Global Burden of Rabies

Rabies Impacts Lives: Physically, Socially, Psychologically & Economically

- Canine Rabies = $124 billion USD Annually – Globally (GARC, 2013)
- Human Life Lost – **most devastating and highest financial cost** (GARC, 2013)
  - Death and future lost earning (directly impacts communities)
- **Next Highest Financial Cost** = Rabies Prophylaxis
  - CDC Estimates $300 million in USA annually (WHO, 2012)
  - Asia Estimates $1.5 billion USD annually for post-exposure (WHO, 2012)
    - 3.8% of their gross income (31 days of income)

Due to the result of growing populations of humans and canines around the world, the Economic cost of rabies along with the burden of human deaths will also continue to grow. *(WHO, 2012)*

Rabies Free / Controlled Regions

Countries and Political Units Currently Rabies Free/Controlled (CDC, 2013)

Region	Countries/Localities
Africa	Cape Verde, Mauritius, Reunion, Sao Tome, and Principe, Seychelles
Americas	North: Bermuda, Saint Pierre and Miquelon Caribbean: Antigua and Barbuda, Aruba The Bahamas, Barbados, Cayman Islands, Dominica, Guadeloupe, Jamaica, Martinique, Montserrat, Netherlands, Antilles, Saint Kitts (Saint Christopher) and Nevis, Saint Lucia, Saint Martin, Saint Vincent and Grenadines, Turks and Caicos, Virgin Islands (UK and US)
Asia and the Middle East	Hong Kong, Japan, Kuwait, Malaysia (Sabah), Qatar, Singapore, United Arab Emirates
Europe[1]	Albania, Austria, Belgium, Corsica, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Gibraltar, Hungary, Iceland, Ireland, Isle of Man, Liechtenstein, Luxembourg, Monaco, Netherlands, Norway (except Svalbard), Portugal, Slovakia, Slovenia, Spain (except Ceuta and Melilla), Sweden, Switzerland, United Kingdom
Oceania[2]	Australia[2] , Cook Islands, Fiji, French Polynesia, Guam Hawaii, Kiribati, Micronesia, New Caledonia, New Zealand, Northern Mariana Islands, Palau, Papua New Guinea, Samoa, Vanuatu

1. Bat lyssaviruses have been reported throughout Europe, including areas that are reportedly free of rabies in other wild mammals.
2. Most of Pacific Oceania is reportedly “rabies-free”, with the exception of Australia, where lyssaviruses in bats have been reported, as well as fatal human rabies cases.

Rabies Free / Controlled Regions

In order for a country to be identified as rabies free, or rabies controlled, per the OIE the following provisions must be achieved:

- ✓ The disease is notifiable (reported to appropriate authorities).
- ✓ Effective disease surveillance has been implemented and is in operation.
- ✓ Regulatory measures for control and prevention have been implemented. This is to also include effective importation procedures.
- ✓ Two years free from having an indigenous case of rabies in animals and man. It is noteworthy to mention that isolating Australian or European Bat Lyssavirus would not affect this status.

No imported case of carnivores outside of the quarantine station has been confirmed with rabies for the previous six months.

Rabies Free / Controlled Regions

The OIE specifically outlines the following recommendations in order to ship dogs and cats into rabies-free regions from countries that are considered infected with rabies:

- ✓ Veterinary authorities must provide an international veterinary certificate that confirms the animal has not shown clinical signs of rabies within the past 48 hours of shipment. The animals must be permanently identified (such as tattoo or microchip) and that identifier must be listed on said certificate.
- ✓ The animal must be vaccinated for rabies, and received not less than six months or more than one year prior to shipment. The initial rabies vaccine should have been administered while the animal was at least 3 months old in age. The vaccine is to be from an inactivated virus vaccine or from a recombinant vaccine that expresses the rabies virus glycoprotein.
- ✓ The animal must be subjected to an antibody test not less than three months or more than twenty four months prior to shipment. The antibody test must reflect that the animal is carrying an antibody response of at least 0.5 IU/mL, which meets WHO recommendations. Animals that have not been vaccinated following the above noted criteria, the importing country can require the animal be placed in quarantine at a quarantine location within that countries territory for six months.

Recommendation & Provisions: Strictly Enforced. Failure to adhere to these guidelines can, and has, resulted in euthanasia of the animal.

Field - Experience

GLOBAL ALLIANCE FOR RABIES CONTROL

Global Alliance For Rabies Control

→ Established 2006/2007: Rabies Experts and Scientific Stakeholders – ARC UK Non-Profit Charity Number SC037112 (GARC, 2014)

→ 2007 Established GARC – US 501c3 Non-Profit (GARC, 2014)

→ Non-Government Entity (NGO)

→ 2007 Established Partners for Rabies Prevention (PRP) (GARC, 2014)

→ Mission: One day eliminating human deaths from rabies and to relieve the burden of rabies in animals, especially dogs. (Stukey, 2012)

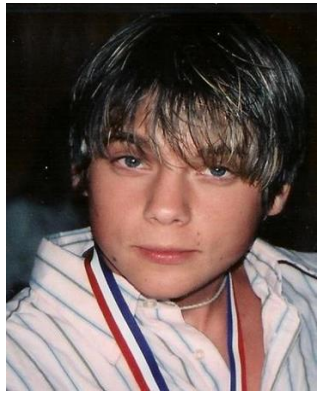
→ World Rabies Day Campaign

As we are aware that rabies is 100% preventable in humans, GARCs vision is "a world free of human rabies" *(GARC, 2014)*

Field - Experience

Zach Jones Memorial Fund

→ Non-Profit 501c3 established 2006
→ Established by Larry & Connie Jones of Humble Tx.
→ Promote Rabies Education
→ Zachary 'Zach' Ross Jones
→ Raise Fund for Rabies Awareness, Early Detection and Scholarships to Graduating Seniors at Atascocita High School



April 29, 1990 - May 12,2006

Field - Experience

Grant Proposal:

Project Intent: Secure 50% of Funds to Promote Rabies Awareness - Relationship between Bats and Rabies (their role in transmitting Rabies

Target Audience: Adolescents

Objective: Educate: What is Rabies, How to Prevent It, and What To D In the Event of and Exposure / Possible Exposure.

Goal: 1 Million Adolescents

Design: PSA & Short Video (English and Spanish)

Proposal Developed and Submitted through GARC
Immediately Accepted by ZJMF….

'ZJMF' Mission: Strives to raise funds in order to assist with educational awareness, early detection, and ultimately the cure for rabies" *(ZJMF, 2014).*

ZACH JONES MEMORIAL FUND
ACADEMICS SPORTS CHARACTER

Field - Experience

Zach 'Z' Jones US Rabies Education Proposal

19"X25" Panel Size Poster/4 Color Process Inks/80# Gloss Coated Text Paper	3,711.04
Envelopes for shipping	2,237.44
Non-profit Postage	743.31
Including DVD /Computer Compatible Video in Packet for Mailing	4,600
Copying of DVD/Computer Compatible Video (Includes protective jacket)	20,700
Electronic Survey (GARC will handle logistics & statistics)	400
Poster Design (Handled by GARC & Independent Graphic Design Artist)	700
Cost per packet that is mailed out comes to	1.06
Program design, coordination and implementation	12,000
Administration 3%	1,353
Total	**$46,445**

ZJMF immediately accepted proposal and submitted check for $18,000.

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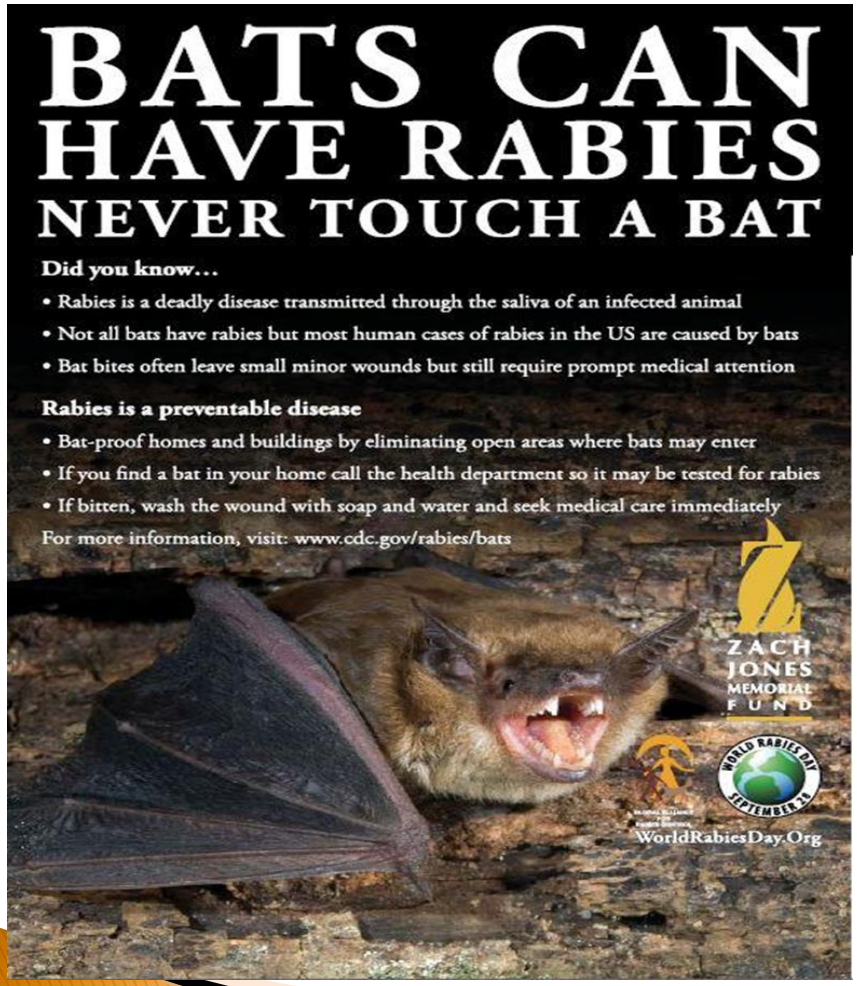
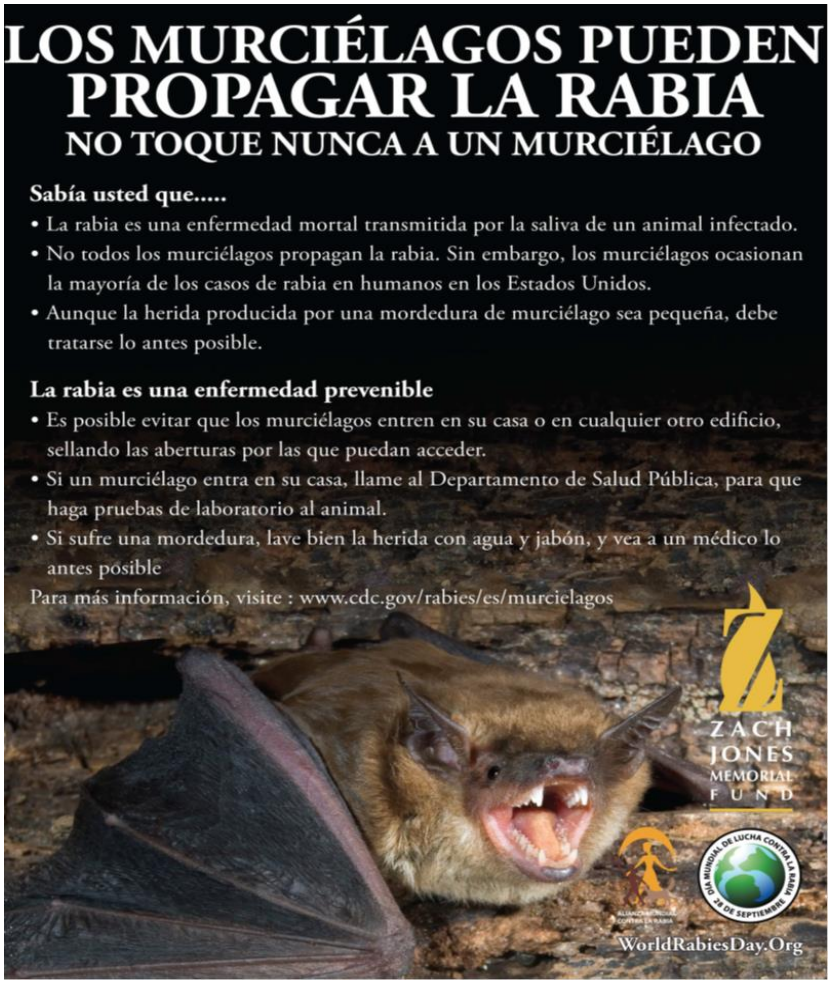
ACADEMICS SPORTS CHARACTER

Field - Experience

PSA was not to allow for interpretations and questioning as topic was critical!
Direct and factual with limited space.





Small working group: GARC and CDC, Included experts, translator, graphic designer and scientists.

Field - Experience

- PSA "Bats Can Have Rabies' Finalized: Immediate Global Distribution
  GARC Websites, List-serves and Social Media Accounts
- USA Outreach Campaign to SPHV
  No Shipping or Printing $
  Spanish and English (11"x17" and 18"x24")
  Developed Request Form and Handled Distribution Logistics
  PSA Distributed: Maine, Florida, North Carolina, Kentucky,
  Minnesota and Arizona

⇓

Video Segment of the ZJMF Grant Immediately Ensued

"Zach Jones is defined not by the way he died, but by the way he lived" *(zachjonesmemorial.org)*

ZACH JONES MEMORIAL FUND
ACADEMICS SPORTS CHARACTER

Field - Experience

Bats and Rabies Video

Plan of Action: Mirror PSA → What is Rabies, How to Prevent It, and What To Do In the Event of and Exposure or Possible Exposure **PLUS** Educate on the Importance of Bats in our Ecosystem.

Step 1: Solicit and Identify Production Team
Step 2: Outline Project Goals and Secure Quote - $
Step 3: Finalize Contract w/ Production Team – Knowlera Media, LLC
Step 4: Arrange for 50% of Invoice
Step 5: Consultation w/ Knowlera: Project Intent, Objective, Goal & Audience

- Adolescence
- Short Video (~ 12 minutes)
- Bats and Rabies
- Outline Discussion Topics

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Field - Experience

GARC 2 Minute Rabies Video.mp4

Bats and Rabies Video

Step 6: Topics were broke out to video segments and script writing began
Step 7: Narrators and expert Identified
Step 8: Identify and Secure Photos, B-Roll Footage, etc…
Step 9: Identify Film Location (Bat Conservation International, Austin Texas)
Step 10: Travel Dates, Plans and Time Line Outlined

Outlined Video Segments:

- Segment 1: Introduction to Rabies
- Segment 2: About Rabies (general scientific information)
- Segment 3: Rabies and Bats
- Segment 4: Preventing Exposures
- Segment 5: Exposures
- Segment 6: How Bats Benefit The Ecosystem
- Segment 7: Closing

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Many hours and edits later…. 2 films completed – 7:22 minutes (for private distribution) and 2:00 minutes (for internet distribution).

Full Video: https://www.youtube.com/watch?v=Zjg_FHqlFnU

Field - Experience

Pfizer Animal Health (Zoetis)

Mass Vaccine Distribution Initiative

Project Intent: Mass donation: animal rabies vaccine. Distributed through GARC across the USA.
- Promote vaccine incentives & increase involvement of the WRD.

Target Groups: Companion Animals for Adoption, Feral Felines and Low-Income Families.

Objective: 250 Doses of Animal Rabies Vaccines Per Organization

Field - Experience

Pfizer Animal Health (Zoetis)

Step 1: Solicit Proposal to Distributor – Pfizer Animal Health
Step 2: Build Team within PA to handle Internal Logistics
Step 3: Develop Application, Upload to GARC Websites, Social Media and List-serves
Step 4: Market Application
Step 5: Review Applicants and Build Data Base of Accepted Applicants
Step 6: Submit Database to Pfizer Animal Health for Packaging and Distribution

Outcomes:

Distributed Vaccine to 32 US States
Vaccinated 250,000 + animals (Companion, Feral Felines and Limited Food Animal and Equines).
Increased Awareness on the WRD Campaign & Physical Involvement.

Las Cruces, NM: Adoption Day and Low-Income Rabies Clinic (Stukey, 2014)

Virus Testing Methodologies

Testing Crucial for Public Health Management:
- Testing Animals for Travel
- Establishing Herd Immunity
- Determine Vaccine Efficacy

Public health concern for countries considered infected with rabies & rabies-free regions.

Testing Crucial for Potential Human Rabies Subject

Testing Crucial for Maintaining 'Adequate Protection'
- Rabies Laboratory Staff
- Veterinarians and Staff
- Animal Control Officers
- Immunocompromised Subject

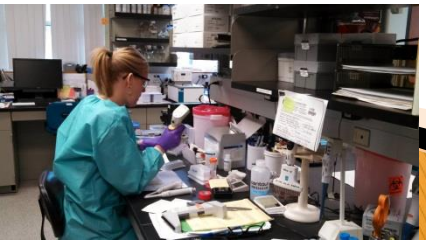


Photo by:
Susan Moore

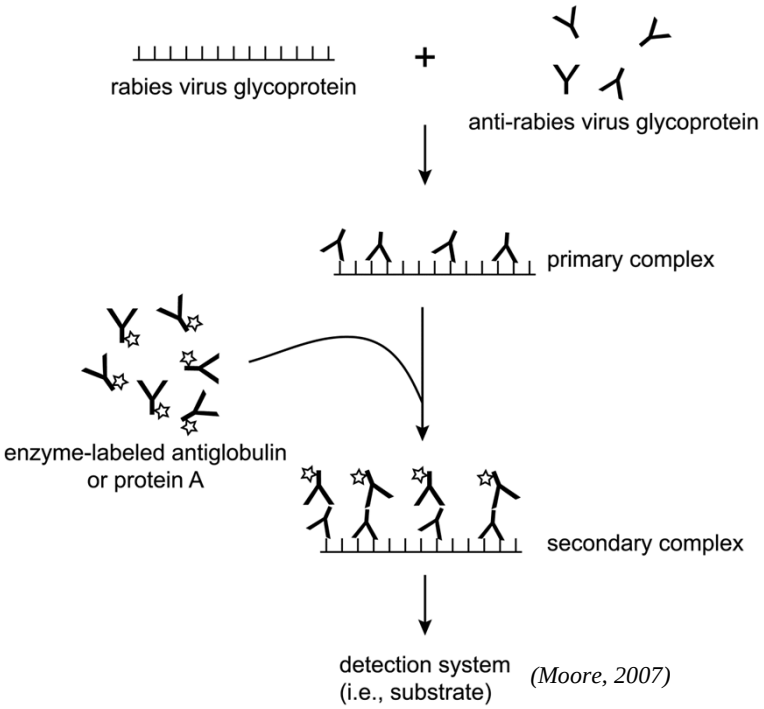
WHO Recommendation: 0.5 IU/mL
Immune Response for Adequate Protection

Virus Testing Methodologies

RFFIT, FAVN and MNT
↓

Testing Methodologies – Measuring Immune Response:

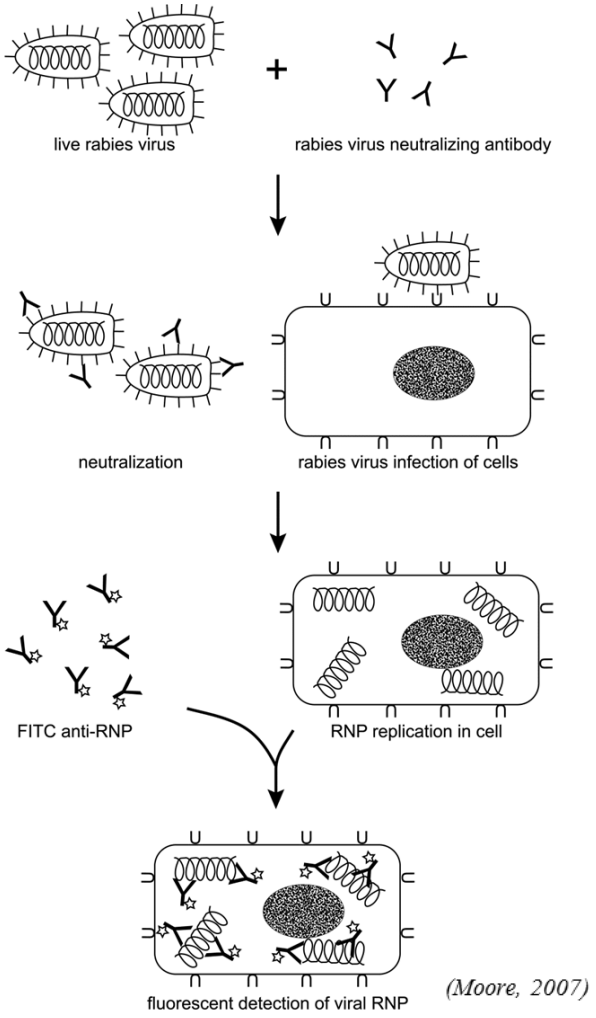
- Antigen-Binding Assay



(Moore, 2007)

↑
ELISAs and IFAs

- Virus-Neutralizing Assay



(Moore, 2007)

Virus Testing Methodologies

Virus-Neutralizing Assay vs Antigen-Binding Assay

Similarities – YES: Both test are measuring an immune response

Differences – YES!

- Virus Neutralization → Measures Neutralizing Antibody (Is Virus Escaping Neutralization…)
- Antigen-Binding → Measures Antigen Binding Antibody (detected, quantified, & characterized by their ability to bind to various rabies virus antigens (Moore, 2013).

Important Note: results will not be consistent between subjects for reasons of Ig subclasses, various affinities, neutralizing abilities and unique polyclonal responses (Moore, 2013).

As these two testing methodologies differ in their identifications and measurements; results should not be equally comparable to one another (Moore, 2013).

» **Recall**: WHO Recommendation: 0.5 IU/mL ⇨ RFFIT and MNT, 1978

Assigning the same cut-off level for both test methods will never result in agreement for all individuals. The relationship of RFFIT and ELISA results over time points post vaccination, … demonstrates further that one cut-off is not appropriate *(Susan Moore, 2013).*

Capstone

Investigating the Performance of a Rabies ELISA Test

Zoetis - SERELISA® Rabies Ab Mono Indirect ELISA

Long-term Objectives: 1) Investigate performance to determine adequate immune response levels to assess potential differing cut-off values in human samples. 2) Establish Standardization Curves for different reference sera.

Step 1: Consult: KSU Director
Step 2: Consult: Zoetis Director
Step 3a: USDA Permit (#VB-139848)
Step 3b: KSU IRB (#7012)
Step 4: Project Protocol
Step 5: Receive the Kits…

* WHO Recommendation = 0.5 IU/mL

Zoetis is a global leading animal health company.

Capstone

(Zoetis, 2014)

(Google Images: 96 well-plate)

Reference Sera	IU/mL	Label
OIE	6.7	Batch 3
WHO-1	59.0	Lot R3 US Standard
WHO-2	30.0	RAI
KSU	59.0	TRP-1

⇓

	OIE	WHO-1	WHO-2	KSU
IU/mL	6.7	59.0	30.0	59.0 → 17.0
Dilution 1	6.7	5.9	6.0	6.0
Dilution 2	2.23	1.97	2.0	2.0
Dilution 3	0.67	0.59	0.6	0.6
Dilution 4	0.447	0.39	0.4	0.4
Dilution 5	0.223	0.197	0.2	0.2
Dilution 6	0.067	0.059	0.06	0.06
Dilution 7	0.0223	0.0197	0.02	0.02

Capstone

PLATE 10 (1st Half – Set Up)

	1	2	3	4	5	6
A	N 1:10	N 1:10	WHO1 1:300,000	WHO1 1:300,000	KSU Ref #1 1:100	KSU Ref #1 1:100
B	P 1:10	P 1:10	OIE 1:100	OIE 1:300	KSU Ref #2 1:100	KSU Ref #2 1:100
C	WHO1 1:1,000	WHO1 1:1,000	OIE 1:1,000	OIE 1:1,500	KSU Ref #3 1:100	KSU Ref #3 1:100
D	WHO1 1:3,000	WHO1 1:3,000	OIE 1:3,000	OIE 1:10,000	KSU Ref #4 1:100	KSU Ref #4 1:100
E	WHO1 1:10,000	WHO1 1:10,000	OIE 1:30,000	WHO2 1:500	KSU Ref #1 1:200	KSU Ref #1 1:200
F	WHO1 1:15,000	WHO1 1:15,000	WHO2 1:1,500	WHO2 1:5,000	KSU Ref #2 1:200	KSU Ref #2 1:200
G	WHO1 1:30,000	WHO1 1:30,000	WHO2 1:7,500	WHO2 1:15,000	KSU Ref #3 1:200	KSU Ref #3 1:200
H	WHO1 1:100,000	WHO1 1:100,000	WHO2 1:50,000	WHO2 1:150,000	KSU Ref #4 1:200	KSU Ref #4 1:200

↑ Plate Scheme

Expected Results →

(Google Images: 96 well-plate)

	1	2	3	4	5	6
A	N 1:10	N 1:10	0.0197 IU/ml	0.0197 IU/ml	0.15 IU/ml	0.15 IU/ml
B	P 1:10	P 1:10	6.7 IU/ml	2.23 IU/ml	2.8 IU/ml	2.8 IU/ml
C	5.9 IU/ml	5.9 IU/ml	0.67 IU/ml	0.447 IU/ml	0.5 IU/ml	0.5 IU/ml
D	1.97 IU/ml	1.97 IU/ml	0.223 IU/ml	0.067 IU/ml	0.1 IU/ml	0.1 IU/ml
E	0.59 IU/ml	0.59 IU/ml	0.0223 IU/ml	6 IU/ml	7.5 IU/ml	7.5 IU/ml
F	0.39 IU/ml	0.39 IU/ml	0.2 U/ml	0.6 IU/ml	1.4 IU/ml	1.4 IU/ml
G	0.197 IU/ml	0.197 IU/ml	0.4 IU/ml	0.2 IU/ml	0.25 IU/ml	0.25 IU/ml
H	0.059 IU/ml	0.059 IU/ml	0.06 IU/ml	0.02 IU/ml	0.05 IU/ml	0.05 IU/ml

Capstone

Kit Performance: Indirect ELISA Performed According to Kit Insert

Final Step:

Automated plate reader: Optical Density (OD) measured bichromatically (450 and 630 nm). Data submitted to Zoetis for internal review and interpretation.

Outcomes:

1st Run: WHO-1 and WHO-2 serial dilutions too similar in results; no other detailed points were noted. → Plate scheme updated serial dilutions for Plate 1, Plate 2 remain as is.

(Google Images: 96 well-plate)

Plate 1 Scheme →

	1	2	3	4	5	6	7	8	9	10	11	12
A	N 1:10	N 1:10	OIEa 1:30,000	OIEa 1:30,000	WHO1 1:150,000	WHO1 1:300,000	WHO2 1:75,000	WHO2 1:150,000	KSU 1:150,000	KSU 1:300,000	OIEa 1:30,000	OIEa 1:30,000
B	P 1:10	P 1:10	KSU Ref#1 1:100	KSU Ref#1 1:100	KSU Ref#2 1:100	KSU Ref#2 1:100	KSU Ref#3 1:100	KSU Ref#3 1:100	KSU Ref#4 1:100	KSU Ref#4 1:100	WHO1 1:150,000	WHO1 1:300,000
C	OIEa 1:100	OIEa 1:100	WHO1 1:500	WHO1 1:1,000	WHO2 1:250	WHO2 1:500	KSU 1:500	KSU 1:1,000	OIEa 1:100	OIEa 1:100	WHO1 1:500	WHO1 1:1,000
D	OIEa 1:300	OIEa 1:300	WHO1 1:1,500	WHO1 1:3,000	WHO2 1:750	WHO2 1:1,500	KSU 1:1,500	KSU 1:3,000	OIEa 1:300	OIEa 1:300	WHO1 1:1,500	WHO1 1:3,000
E	OIEa 1:1,000	OIEa 1:1,000	WHO1 1:5,000	WHO1 1:10,000	WHO2 1:2,500	WHO2 1:5,000	KSU 1:5,000	KSU 1:10,000	OIEa 1:1,000	OIEa 1:1,000	WHO1 1:5,000	WHO1 1:10,000
F	OIEa 1:1,500	OIEa 1:1,500	WHO1 1:7,500	WHO1 1:15,000	WHO2 1:3,750	WHO2 1:7,500	KSU 1:7,500	KSU 1:15,000	OIEa 1:1,500	OIEa 1:1,500	WHO1 1:7,500	WHO1 1:15,000
G	OIEa 1:3,000	OIEa 1:3,000	WHO1 1:15,000	WHO1 1:30,000	WHO2 1:7,500	WHO2 1:15,000	KSU 1:15,000	KSU 1:30,000	OIEa 1:3,000	OIEa 1:3,000	WHO1 1:15,000	WHO1 1:30,000
H	OIEa 1:10,000	OIEa 1:10,000	WHO1 1:50,000	WHO1 1:100,000	WHO2 1:25,000	WHO2 1:50,000	KSU 1:50,000	KSU 1:100,000	OIEa 1:10,000	OIEa 1:10,000	WHO1 1:50,000	WHO1 1:100,000

Expected Results →

	1	2	3	4	5	6	7	8	9	10	11	12
A	N 1:10	N 1:10	0.0223 IU/ml	0.0223 IU/ml	0.0197 IU/ml	0.0197 IU/ml	0.02 IU/ml	0.02 IU/ml	0.0197 IU/ml	0.0197 IU/ml	0.0223 IU/ml	0.0223 IU/ml
B	P 1:10	P 1:10	15.0 IU/ml	15.0 IU/ml	2.8 IU/ml	2.8 IU/ml	0.5 IU/ml	0.5 IU/ml	0.1 IU/ml	0.1 IU/ml	0.0197 IU/ml	0.0197 IU/ml
C	6.7 IU/ml	6.7 IU/ml	5.9 IU/ml	5.9 IU/ml	6 IU/ml	6 IU/ml	5.9 IU/ml	5.9 IU/ml	6.7 IU/ml	6.7 IU/ml	5.9 IU/ml	5.9 IU/ml
D	2.23 IU/ml	2.23 IU/ml	1.97 IU/ml	1.97 IU/ml	2 IU/ml	2 IU/ml	1.97 IU/ml	1.97 IU/ml	2.23 IU/ml	2.23 IU/ml	1.97 IU/ml	1.97 IU/ml
E	0.67 IU/ml	0.67 IU/ml	0.59 IU/ml	0.59 IU/ml	0.6 IU/ml	0.6 IU/ml	0.59 IU/ml	0.59 IU/ml	0.67 IU/ml	0.67 IU/ml	0.59 IU/ml	0.59 IU/ml
F	0.447 IU/ml	0.447 IU/ml	0.39 IU/ml	0.39 IU/ml	0.4 IU/ml	0.4 IU/ml	0.39 IU/ml	0.39 IU/ml	0.447 IU/ml	0.447 IU/ml	0.39 IU/ml	0.39 IU/ml
G	0.223 IU/ml	0.223 IU/ml	0.197 IU/ml	0.197 IU/ml	0.2 IU/ml	0.2 IU/ml	0.197 IU/ml	0.197 IU/ml	0.223 IU/ml	0.223 IU/ml	0.197 IU/ml	0.197 IU/ml
H	0.067 IU/ml	0.067 IU/ml	0.059 IU/ml	0.059 IU/ml	0.06 IU/ml	0.06 IU/ml	0.059 IU/ml	0.059 IU/ml	0.067 IU/ml	0.067 IU/ml	0.059 IU/ml	0.059 IU/ml

Capstone

Validation: > 0.95

Outcomes, 1st RUN: Good, consistent & clean - excess of variation detected between few of the serial dilutions than projected. Resulted in one reference sera validation.
Values = 86.2, 97.2, 94.8, 83.8, 91.7 and 88.3 for Plate 1 and 92.6, 94.3, 87.9, 81.1, 85.1 and -19.5 for Plate 2.

⇨ Re-run Plate 1 / Plate 2: Remove some reference dilutions & cut WHO-1 and WHO-2 dilutions in half

Outcomes, 2nd RUN: Results reflected WHO-1 and WHO-2 = improved results. Values = 93.2, 94.6, 93.1, 86.9, and 93.5 for Plate 1 and 96.9, 94.8, 89.0, 84.4, 90.9 and -13.6 for Plate 2. (Concern did not weigh heavily on the -13.6…).
Second validation obtained.

⇨ Slightly alter next run. Discussed: plates in parallel with multiple technicians, additional plate washings, and running duplicate reference sera in triplicate.
→ Identified washing steps: manually vs machine.
** Run duplicate sera in triplicate and use automated washer for wash steps.

Capstone

Plate Scheme: '3rd' Run:

(Google Images: 96 well-plate)

Manual Wash | Automated Wash | Manual Wash

	1	2	3	4	5	6	7	8	9	10	11	12
A	N 1:10	N 1:10	WHO1 1:150,000	WHO1 1:150,000	WHO1 1:500	WHO1 1:500	WHO2 1:750	WHO2 1:750	WHO1 1:500	WHO1 1:500	WHO2 1:250	WHO2 1:250
B	P 1:10	P 1:10	WHO2 1:250	WHO2 1:250	WHO1 1:1,500	WHO1 1:1,500	WHO2 1:2,500	WHO2 1:2,500	WHO1 1:1,500	WHO1 1:1,500	WHO2 1:750	WHO2 1:750
C	WHO1 1:500	WHO1 1:500	WHO2 1:750	WHO2 1:750	WHO1 1:5,000	WHO1 1:5,000	WHO2 1:3,750	WHO2 1:3,750	WHO1 1:5,000	WHO1 1:5,000	WHO2 1:2,500	WHO2 1:2,500
D	WHO1 1:1,500	WHO1 1:1,500	WHO2 1:2,500	WHO2 1:2,500	WHO1 1:7,500	WHO1 1:7,500	WHO2 1:7,500	WHO2 1:7,500	WHO1 1:7,500	WHO1 1:7,500	WHO2 1:3,750	WHO2 1:3,750
E	WHO1 1:5,000	WHO1 1:5,000	WHO2 1:3,750	WHO2 1:3,750	WHO1 1:15,000	WHO1 1:15,000	WHO2 1:25,000	WHO2 1:25,000	WHO1 1:15,000	WHO1 1:15,000	WHO2 1:7,500	WHO2 1:7,500
F	WHO1 1:7,500	WHO1 1:7,500	WHO2 1:7,500	WHO2 1:7,500	WHO1 1:50,000	WHO1 1:50,000	WHO2 1:75,000	WHO2 1:75,000	WHO1 1:50,000	WHO1 1:50,000	WHO2 1:25,000	WHO2 1:25,000
G	WHO1 1:15,000	WHO1 1:15,000	WHO2 1:25,000	WHO2 1:25,000	WHO1 1:150,000	WHO1 1:150,000			WHO1 1:150,000	WHO1 1:150,000	WHO2 1:75,000	WHO2 1:75,000
H	WHO1 1:50,000	WHO1 1:50,000	WHO2 1:75,000	WHO2 1:75,000	WHO2 1:250	WHO2 1:250						

Outcome - Pending: Analysis & interpretation by Zoetis still pending. As I continue to move forward in this project with Zoetis and KSU, we are all optimistic final goals & objectives will result in success.

* WHO Recommendation = 0.5 IU/mL

Recommendations

"As human and canine population continue to grow, the cost and economic burden will also continue to grow" *(WHO, 2012)*

- Additional efforts for vaccine, ORV & contraceptive programs for free roaming K-9s
- Ensuring prompt wound management & proper PEP and RIG
- Further use of effective blue prints & international advocacy
- Implementing national programs w/ effective surveillance & control efforts
- Minimize human exposures to infected animals

In Reference to Research

- Additional science based studies for development of recommendation
- Well designed studies to further purse:
  - Potential virus shedding in milk
  - PEP for animals,
  - Viral shedding in livestock
  - Ecology of rabies in wildlife.

(Compendium of Animal Rabies Prevention and Control, 2011).

Take – Home Messages…

I want you to leave here today with two things in reference to rabies – if you leave here today with nothing else, please take home these messages ...

1) Rabies is 100% preventable in humans by proper administration of prophylaxis!

2) In any event you receive a bite / scratch, immediately wash the wound for several minutes w/ soap and water (decreases the viral load!). Or, if you come in contact (or are in the presence of) a bat please report it!

PS: Always be a responsible pet owner and VACCINATE!

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QUESTIONS….?

Photo provided by
Benjamin R McKenna, MPH

“NEVER DOUBT THAT A SMALL GROUP OF COMMITTED PEOPLE CAN

CHANGE THE WORLD.

INDEED, IT IS THE ONLY THING THAT EVER HAS.”

-DR. MARGARET MEAD

Dr. Margaret Mead: American Cultural Anthropologist
(December 16, 1901 – November 15, 1978)

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