

RABIES VACCINE STRATEGIES

Concepts of Rabies Prophylaxis for the Caving Community

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Rabies is a rapidly progressive and fatal viral infection of the central nervous system (Figures 1 and 2). The causative agent is a bullet-shaped virus (Figure 3) belonging to the genus *Lyssavirus* (from the Greek word *lyssa*, meaning frenzy or madness) of the family *Rhabdoviridae* (from the Greek word *rhabdos*, meaning rod).

Although primarily a disease of animals, humans may also be infected. Thus, rabies is a zoonotic disease, a zoonosis being an infectious disease that can be transmitted between vertebrate animals and humans. The most common mode of transmission of rabies virus is the bite of a rabid animal; however, less common non-bite routes of transmission, though rare, are also known.

With the exception of rare cases following organ or corneal transplants originating from misdiagnosed donors who had died of rabies, there have been no confirmed reports of human-to-human transmission of rabies virus. Nevertheless, the possibility remains an important consideration in dealing with friends, family, and health-care workers who may have been in close contact with a rabies victim and perhaps exposed to potentially infectious bodily fluids (e.g., to saliva through shared drinks or eating utensils). Such concern also extends to mortuary personnel.

While rabies is global in distribution, its public health impact varies considerably from one country to another. Most notable in this regard is the extent to which disease has been controlled in canine populations, since dogs are the major source of human rabies on a global basis. Worldwide, an estimated 55,000 human deaths from rabies—the actual figure may be significantly higher—are believed to occur annually, principally following bites from rabid dogs. Each year, approximately four million people in more than 80 countries



Figure 1- This photograph of a human rabies victim being offered a glass of water illustrates the severe muscle spasms, choking, and gagging that characterize a terrifying episode of hydrophobia (fear of water). This is one of the common manifestations of rabies infection in humans. In fact, human rabies is often referred to simply as hydrophobia. Courtesy of Professor David A. Warrell (©). Centre for Tropical Medicine and Infectious Diseases, University of Oxford.

Abbreviations used in this article

ACIP	Advisory Committee on Immunization Practices
CDC	Centers for Disease Control and Prevention
HRIG	human rabies immune globulin
IU	International Unit
mL	milliliter
NSS	National Speleological Society

(including an estimated 11,000 to 40,000 in the United States) require post-exposure rabies prophylaxis.

A Brief Overview of Sylvatic Rabies in the United States

Although all mammals are susceptible to rabies infection, species-specific importance in the overall scheme of rabies epidemiology/epizootiology is highly variable. In the United States, rabies is maintained only in wildlife populations. The canine variant of virus (including the canine/coyote variant) no longer circulates in this country; although, continued surveillance along the U.S./Mexican border is important. Several terrestrial species—notably raccoons, skunks, and foxes—are natural reservoirs of this disease, maintaining a pool of virus in nature. The relative public health significance of these mammalian reservoirs (or *rabies-vector species*) varies considerably in different parts of the country.¹

As canine rabies was brought under control and the attention of public health authorities shifted to wildlife, it became apparent that rabies was compartmentalized in both geographic and species-specific fashion. That is to say, the significance of different species in *sylvatic* (wildlife) rabies cycles was observed to vary across the country. It is now known that in a particular region in which rabies is prevalent, cases will predominate in a single reservoir species—so-called *single-species involvement*—with low numbers of cases in other species in the same area and low numbers of cases in this reservoir species in different areas (Figure 4). For example, skunk rabies stretches primarily as a broad band across the Midwest and the eastern seaboard is plagued by an expanding epizootic (animal epidemic) of rabies in raccoons. Sporadic cases of rabies in non-reservoir species (i.e., domestic animals or other species of wildlife) are generally due to spillover from infection in the geographic reservoir.

Superimposed on this terrestrial rabies cycle is a geographically widespread non-terrestrial cycle in insectivorous bats. Rabies in insectivorous bats is reported throughout the contiguous United States (Figure 5). In some states, various species of bats are the only identified reservoir of virus. In 2007, bats accounted for 27.2% (1,973) of all animal rabies cases reported by public health facilities in this country—up

¹Oral vaccination programs have significantly reduced the number of reported cases of animals infected with either the Arizona or Texas gray fox variant of virus.

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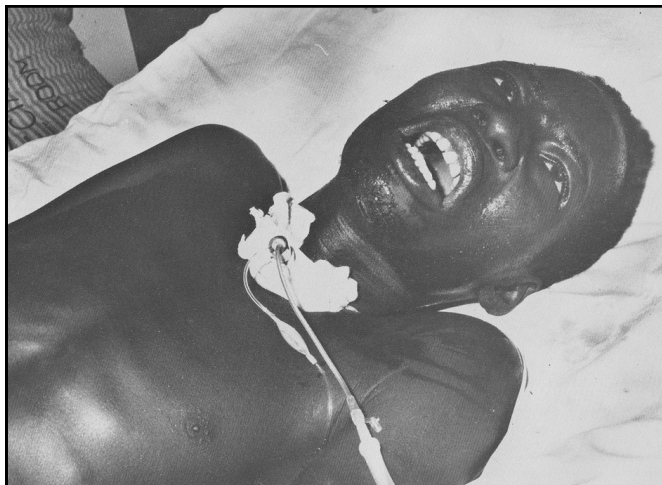


Figure 2 - A sense of impending doom, terrifying hallucinations (illustrated), and an overwhelming fear often grip human rabies patients and, in general, the disease remains one of terror, agony, and hopelessness. Courtesy of Professor David A. Warrell (©).

from 24.4% or 1,692 reported cases in 2006—making bats the second most commonly reported rabid animal (behind raccoons). Obviously, such statistics are influenced by both the frequency of human encounters with bats and the number of submissions made to testing facilities. Certainly, many more cases of rabies in wild populations go unreported. However, the prevalence of rabies infection in suspect submissions made to public health laboratories is considerably higher than that in wild bat populations, which remains quite low.

Naturally occurring rabies infection in insectivorous bats was first identified in the early 1950s, prompting concern that these animals might be of special significance in both urban and sylvatic rabies cycles. The discovery of rabies in North American bat populations led to the belief among some early investigators that bats played a pivotal role in the initiation and maintenance of infection in terrestrial species (Figure 6). Favoring such an association, some investigators pointed to the possibility that the seasonal migration of bats could explain the occurrence of enzootic (endemic) rabies in geographically widely separated wildlife populations. Although sporadic transmission of rabies from bats to other animals (both wild and domestic), as well as to humans, does occur—and minor “outbreaks” of bat-origin rabies virus in a small cluster of terrestrial animals have, on very rare occasion, been reported—modern epidemiologic evidence is consistent with the belief that terrestrial and non-terrestrial rabies cycles are largely independent of one another.

Prevalence of Human Rabies in the United States

Human rabies is presently an uncommon condition in the United States. On average, less than three cases are reported annually. In the years following the mid 1950s, the number of reported cases declined markedly. This drastic fall in human disease was directly related to the introduction of canine immunization programs in the late 1940s. Ongoing educational programs, availability of safe and effective pharmaceuticals for post-exposure prophylaxis, and an efficient public health infrastructure have also contributed to the low number of human rabies cases. The scarcity of human deaths, however,

does not mean that rabies is no longer a threat to human health. In fact, tens of thousands of people generally receive post-exposure prophylaxis in this country each year, at costs as high as \$2,000 to \$4,500 per exposure. Moreover, the increasing occurrence of human rabies attributed to variants of virus maintained in bat populations suggests that control of the disease in terrestrial animals will not completely eliminate the threat to public health.

Based on the isolation of a characteristic non-terrestrial variant of virus—or, in a limited number of cases, on uncovering a revealing exposure history by epidemiologic investigation—rabies virus of insectivorous-bat origin has been implicated in 96.8% (30/31) of the indigenously acquired human rabies cases in this country since 1995 (note: this includes four cases in June 2004 that were secondary to organ transplants from a misdiagnosed donor who died of bat-origin rabies, but does not include one case in a newly arrived Mexican immigrant who died of bat-origin rabies in March 2008 since infection was likely acquired outside of the United States).

Although a history of having interacted in some manner with a bat or of a bat having been present in a home are not uncommon scenarios in bat-origin human rabies cases, this is not always the case.² Moreover, no distinct history of either a bite or scratch—so-called *cryptic bat rabies*—could be identified in the majority of bat-associated human rabies cases reported in this country in the past quarter century. These figures reflect significant changes in the epidemiology of human rabies in this country in recent decades. Cryptic bat rabies does not imply that a bite or scratch from a rabid bat did not take place, only that no

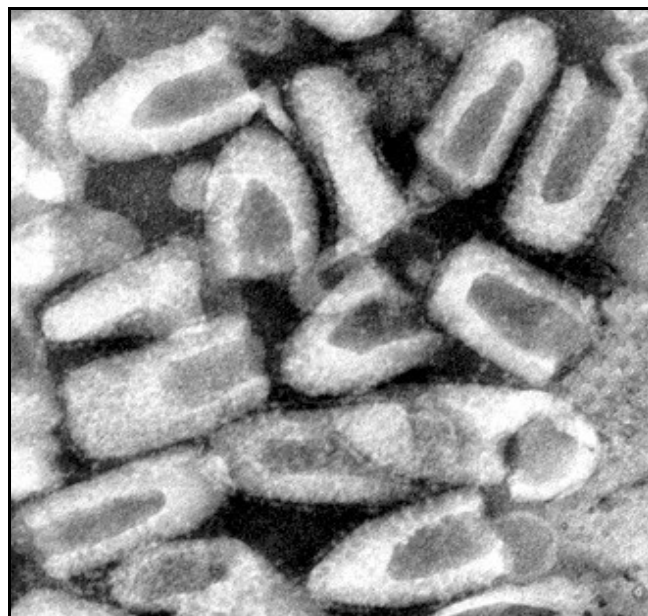


Figure 3 - Characteristic bullet shape of the rabies virus. Magnification approximately $\times 70,000$. Courtesy of Dr. Frederick Murphy, Department of Pathology, University of Texas Medical Branch.

² Such scenarios also often typify the many bat-human encounters each year that do not result in either human exposure or human infection. However, they do often result in the administration of post-exposure prophylaxis.

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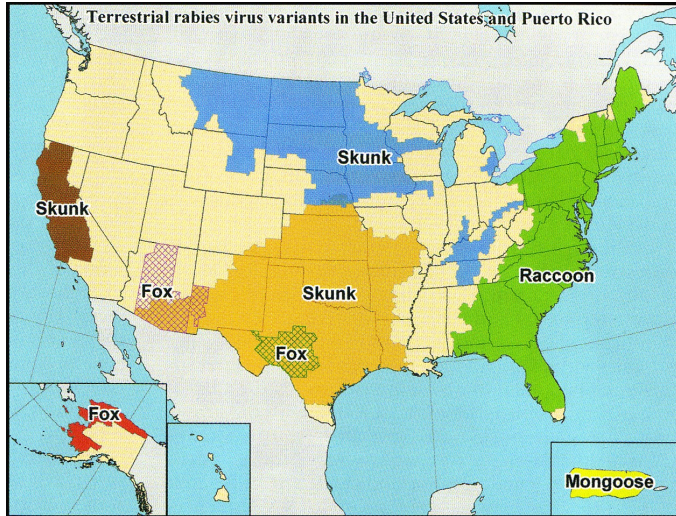


Figure 4 - Distribution of major rabies virus variants among wild terrestrial reservoirs in the United States and Puerto Rico, 2007. Reproduced with permission from Blanton *et al.* (2008).

definitive history of such an exposure could be uncovered.

Reasons for cryptic bat rabies may include several possibilities: 1) fleeting contact with a bat that leaves little or no obvious evidence of a bite or scratch (Figures 7 and 8); 2) an unrecognized encounter with a bat while asleep, unconscious, or otherwise impaired; 3) a minor encounter that happened long enough ago as to have been forgotten—perhaps related to a failure in appreciating the significance of a bat bite or scratch in the first place; 4) inability to obtain an adequate history from a patient due to language barriers, age, or memory lapse/speech impediments secondary to a rabies encephalitis; and 5) indirect and unrecognized secondary transmission of a non-terrestrial variant of virus via a species other than a bat (e.g., a cat infected with a bat variant of rabies virus bites a person).

Exposure of young children or very elderly individuals to a potentially rabid animal can be particularly troublesome since a significant exposure history (especially following a fleeting interaction with a bat) may never come to the attention of an adult or caretaker. The consequences, of course, can be disastrous. In view of the fact that children represent the majority of human

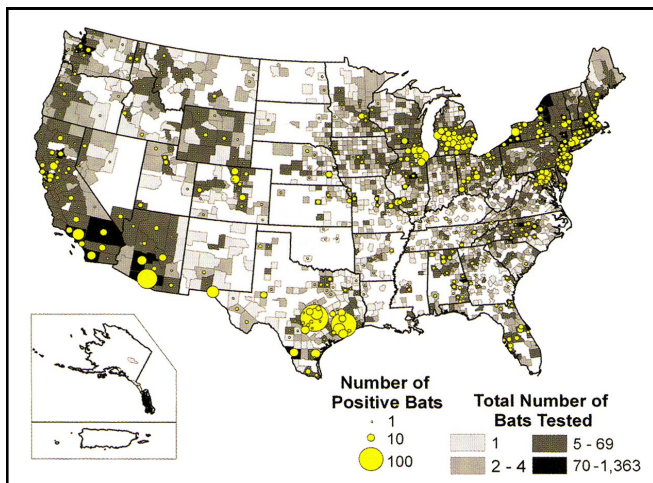


Figure 5 - Reported cases of rabies in bats, by county, 2007. Reproduced with permission from Blanton *et al.* (2008).

rabies cases, as well as being the most common recipients of post-exposure prophylaxis, it is particularly important that they be educated about the potential dangers of handling any wild animal—especially small and seemingly innocuous creatures like bats. Understanding the dangers of handling wildlife and maintaining current rabies vaccinations of pets remain the most important safeguards against the threat of this frightening disease.

Misconceptions about the epidemiology of rabies and the nature of post-exposure prophylaxis abound. Many people, for example, mistakenly believe that medical intervention following potential exposure to rabies can be safely delayed until after the onset of clinical signs or symptoms appear, at which time treatment can be pursued. In fact, post-exposure prophylaxis, if deemed necessary, should be initiated as soon as possible. Once clinical manifestations develop, the disease runs an almost invariably fatal course.³

Of particular concern with respect to human rabies of insectivorous bat origin is the not-uncommon perception that small animals are of only limited significance as disease vectors—the so-called *small vector hypothesis*. While encounters with larger animals usually prompt bite victims to seek medical attention without undue delay, many people consider the relatively minor wounds inflicted by such tiny creatures as bats to be of little public health concern. In spite of widespread attempts at educating the public about the inherent risks of contact with bats, a surprising number of people still do not appreciate the fact that even a minor wound caused by the bite, and possibly scratch, of a rabid bat can serve as a portal for transmission of virus; hence, the importance of consulting with medical professionals following direct physical contact with a bat. As such, it is important to recognize the fact that human deaths from bat-origin rabies virus are not associated with a failure of

³The Milwaukee protocol is a treatment regimen that saved the life of a 15-year-old Wisconsin girl who developed rabies after being bitten on the finger by a bat in 2004. She was only the sixth person known to have survived rabies. At the center of this complex therapeutic regimen is a protocol for placing the patient in a medically induced coma on ventilator support, the aim of which is to hopefully slow down progression of the disease until the body's own immune system can effectively mount an immune response sufficient to combat the virus. In November 2008, a 15-year-old Brazilian boy, who developed rabies following the bite of a vampire bat, had been awakened from his coma and is reported to be a second Milwaukee-protocol survivor; although, his long-term medical status remains in question.* The case of an eight-year-old Colombian girl, who subsequently died of pneumonia remains to be confirmed. Although the overall utility of this medically intensive treatment modality has been criticized by some, the Milwaukee protocol clearly remains of considerable interest; however, issues related to potentially severe neurologic sequelae also remain of significant concern. To date, this protocol, or some variation of it, has been used to treat almost two dozen human rabies patients worldwide. Although it was hoped that the Milwaukee protocol for rabies treatment might have proven to be the magic bullet that has eluded medical research for millennia, this has, so far, turned out not to be the case. Of the various human rabies victims who have been treated with this protocol, none of the others have survived. In considering the reasons behind these failures, medical science is left with the undeniable fact that a multitude of factors are involved, most of them poorly understood at the present time.

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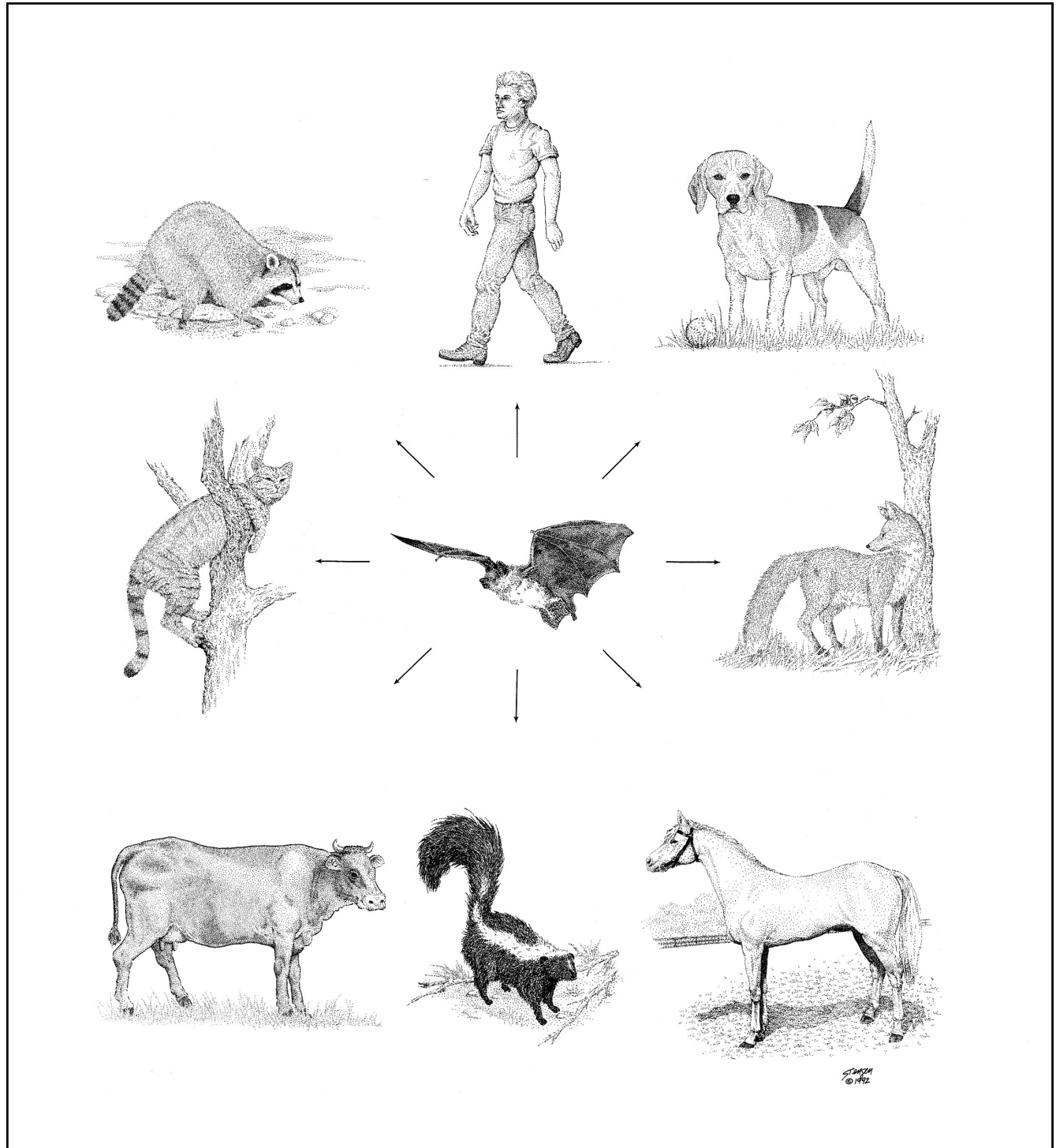


Figure 6 - With the discovery of enzootic rabies infection in insectivorous bat populations, early investigators considered these animals to play a central role in terrestrial rabies cycles. In addition to sporadically infecting domestic animals (and occasionally humans), some researchers believed that bats maintained a wellspring of virus that continually refueled infectious disease cycles in terrestrial reservoir species. Today, this latter aspect of bat rabies is known not to be the case. Reproduced from Brass, D. A. (1994).

post-exposure prophylaxis, but with a failure to seek medical attention in the first place (either because an exposure was not recognized or because it was not considered to be of significant concern).

With respect to validity of the small vector hypothesis, nothing could actually be further from the truth. In fact, there is evidence to suggest that the variant of rabies virus associated with silver-haired bats (*Lasiurus noctivagus*)—and per-

haps eastern pipistrelles (*Pipistrellus subflavus*) as well—might have properties that enhance its transmission following only limited and superficial contact. The majority of human rabies cases in the United States in which bats have been implicated have been attributed to a variant of virus associated almost exclusively with one of these two species (note: following a recent revision in nomenclature, the eastern pipistrelle has been placed in its own genus and is currently referred to as the tri-colored

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Figure 7 - Bat bite on finger. Courtesy of Dr. Cathleen Hanlon, Division of Viral and Rickettsial Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention.

bat, *Perimyotis subflavus*).

In view of an apparent increase in the number of human rabies cases of bat origin in recent decades and because even limited contact with a rabid bat may result in transmission of virus, public health authorities have encouraged a more aggressive policy regarding the need for rabies testing of bats found in homes—even in the absence of overt evidence of a bite, scratch, or other direct contact. This may be of particular importance when a bat is found in proximity to unattended children or pets, people asleep (especially deeply asleep), and impaired individuals (including those under the influence of alcohol or drugs) and the possibility of a bite or scratch cannot be reliably ruled out. Under such circumstances, consideration of post-exposure prophylaxis is recommended, unless prompt testing of the bat rules out any possibility of rabies infection.

In the event of confirmed or suspected direct physical contact with a bat found in the home, every effort should be made to capture the bat, if possible, for submission to a public health laboratory for rabies testing. On the other hand, if contact with humans or domestic animals can be reliably excluded, the bat can be released to the outside. After closing off all avenues of escape except those to the outside, the bat will probably leave on its own. If not, it can be gently captured and then released. To capture a bat in the home, a coffee can, or something similar, can be used to trap it against a wall and a cover then slipped and taped across the front. Of course, adequate precautions, such as

⁴Making suspect animals available for testing, if possible, will help prevent needless post-exposure prophylaxis. This is particularly important at the present time since the availability of rabies vaccine is severely limited while Sanofi Pasteur, one of the two suppliers of rabies vaccine to the U.S., renovates its production facilities in France to comply with the most current requirements of both the U.S. Food and Drug Administration and the French regulatory body. Until dwindling vaccine stockpiles have been replaced, pre-exposure vaccination isn't even available to the general public, regardless of one's risk status. Post-exposure vaccine administration is being carefully monitored at both state and federal levels and a risk assessment being made on a case-by-case basis to ensure appropriate use of vaccine in order to prevent exhaustion of available supplies. It is believed that Sanofi Pasteur's renovated facilities will be approved and back on line by mid to late 2009.

wearing sturdy leather gloves, should be taken to avoid any possibility of incurring direct contact. Alternatively, professionals, such as local wildlife authorities, animal-control officers, or animal rehabilitators, can be contacted to safely remove the animal from a home.⁴ In order to help ensure that the bat is submitted for rabies testing and not accidentally released or otherwise discarded, it is imperative that such workers be made aware of the fact that a possible human exposure is involved.

Pre-Exposure Vaccination

Because many people face a higher-than-average or somewhat unpredictable risk of exposure to rabies virus, a pre-exposure vaccination series is an important safeguard for various individuals. People are most commonly vaccinated against rabies because either their occupation or avocation places them at a relatively higher potential risk than that of the general public for being exposed to this virus. Vaccination consists of three intramuscular injections of vaccine. The first dose is administered on what is defined as day 0; this is followed by an additional dose on day 7 and a final dose on day 21 or 28.

Persons generally considered to be at higher occupational risk of exposure to rabies and who often undergo routine pre-exposure vaccination include veterinarians, veterinary students, veterinary technicians, animal-control officers, animal handlers in zoos, and people who work with wildlife—especially with rabies-vector species—such as animal rehabilitators, wildlife biologists, and others. Pre-exposure vaccination is also very important for professional staff working in rabies research and diagnostic laboratories or vaccine-production facilities. People living in or making extended visits to developing countries, in which canine rabies may be prevalent or where post-exposure prophylaxis with potent and safe pharmaceuticals might not be readily available, may also be candidates for pre-exposure vaccination, especially if they are likely to come into contact with potentially rabid animals (note: a regularly updated and detailed worldwide advisory on recommended travel vaccina-

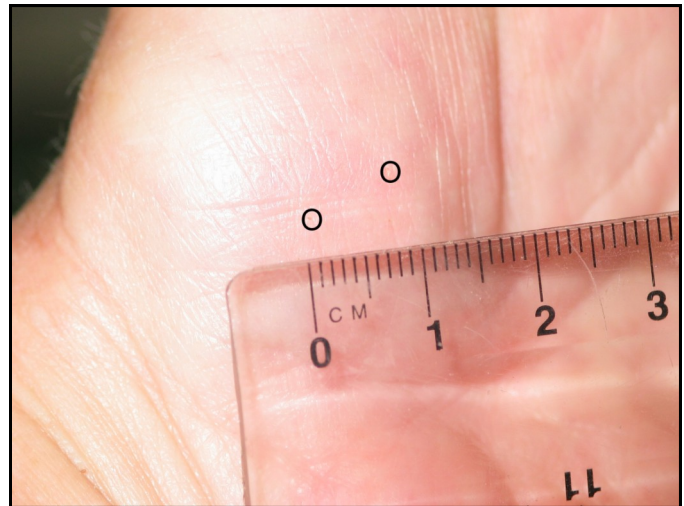


Figure 8 - Bat bite on base of thumb (thenar eminence). Because even the largest of bats in this country have small teeth, puncture wounds from bites may be barely visible. The distance between tooth marks may vary considerably from one species to another and also with the particular teeth involved (e.g., upper and/or lower canines). Courtesy of Barbara Schmidt-French.

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Rabies pre-exposure prophylaxis guide — United States, 2008

Risk category	Nature of risk	Typical populations	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 laboratory workers, cavers, veterinarians and staff, and animal-control and wildlife workers in areas where rabies is enzootic. All persons who frequently handle bats.	Primary course. Serologic testing every 2 years; booster vaccination if antibody titer is below acceptable level.*
Infrequent (greater than population at large)	Exposure nearly always episodic with source recognized. Bite or nonbite exposure.	Veterinarians and animal-control staff working with terrestrial animals 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 areas where rabies is epizootic.	No vaccination necessary.

* Minimum acceptable antibody level is complete virus neutralization at a 1:5 serum dilution by the rapid fluorescent focus inhibition test. A booster dose should be administered if the titer falls below this level.

Table 1 - Recommendations for pre-exposure rabies vaccination and serologic testing in the United States. Reproduced with permission from the *Recommendations of the Advisory Committee on Immunization Practices, Human Rabies Prevention – United States* (2008).

tions, as well as a wealth of additional information of value to traveler health, can be found on the CDC website: <http://wwwn.cdc.gov/travel/destinationList.aspx>). Rabies also presents an unpredictable threat to field soldiers operating in theaters in which the disease is prevalent. Hence, pre-exposure vaccination may be an appropriate consideration for these individuals as well. Finally, anyone whose avocational pursuits (e.g., cave explorers) increase his or her chances of coming into contact with potentially rabid animals should also consider the need for pre-exposure vaccination. Routine pre-exposure vaccination of the general public is not recommended in this country, nor is it necessary to vaccinate people traveling to areas in which animal rabies is not common (Table 1).

The Role of Serology in Rabies Protection Strategies

In clinical practice, immunization with modern cell-culture rabies vaccines is so efficient that determination of an initial rabies titer following administration of a pre-exposure vaccine series (in people who are not immunocompromised) is no longer considered necessary. However, for individuals considered to be at higher-than-average risk of exposure to virus—and especially those at risk of sustaining an unrecognized exposure—regular titer determinations and booster vaccinations, if required, are strongly recommended by public health authorities.

As shown in Table 1, the Advisory Committee on Immunization Practices (ACIP) defines four discrete human populations with respect to rabies risk status: continuous, frequent, infrequent, and rare. Of particular concern in regard to these groups are the differences in pre-exposure vaccine recommendations and suggested serologic testing protocols. The salient features distinguishing these four categories from one another

are related not only to the likelihood of sustaining an exposure, but also to the more ominous possibility of sustaining an unrecognized exposure. In this country, pre-exposure vaccinations are recommended for all groups except that of the general population at large, for whom the risk of exposure to rabies is rare.

In the United States, public health authorities recommend that individuals at *continuous* risk of sustaining an exposure to rabies (including inapparent exposures) be pre-exposure vaccinated, have serology testing every six months, and be given a routine booster dose of vaccine if their serum fails to achieve complete neutralization of virus at a 1:5 serum dilution as measured by the Rapid Fluorescent Focus Inhibition Test. People considered to be at *frequent* risk of exposure are encouraged to be pre-exposure vaccinated, have serology testing every two years, and be given a single booster dose of vaccine if their titer is less than complete viral neutralization at the 1:5 serum dilution (note: if following World Health Organization guidelines, a booster dose of vaccine would be required for individuals in these two high-risk groups if the measured concentration of serum rabies-neutralizing antibodies falls below 0.5 IU/mL; minor differences also exist in recommended serology protocols). The rationale for regularly scheduled titer determinations and booster doses of vaccine, if required, in people considered to be in one of these high-risk groups is mainly one of providing an added level of protection in case of an unrecognized exposure to virus.

There is no requirement for routine serologic testing in individuals who have received a full pre-exposure vaccine series, but who are not included in one of the high-risk groups mentioned above. In such persons—members of the *infrequent* risk group—a potential exposure is likely to be recognized and post-exposure prophylaxis would be administered

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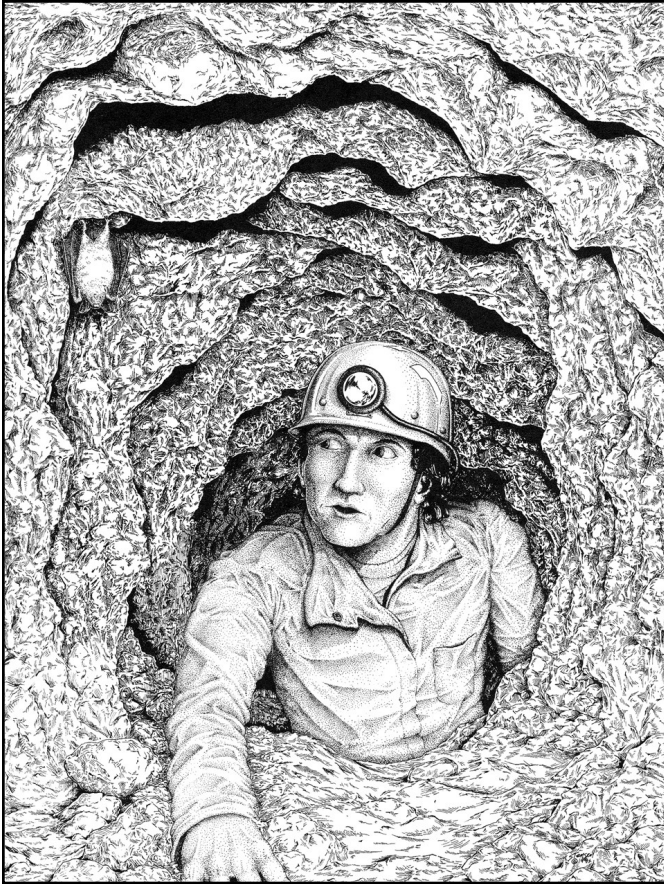


Figure 9 - Bats are commonly encountered in underground passageways. Cavers should move quickly past them and care should be taken to avoid any undue disturbance. Reproduced from Brass, D. A. (1994).

as required (and as specified for an individual who has previously received a full pre-exposure vaccine series).

The Question of Increased Risk

Wildlife rehabilitators and especially members of the caving community sometimes fail to appreciate how public health authorities regard their risk status. In view of the fact that rabies has the highest case fatality rate—essentially 100%—of any known infectious disease, cavers and wildlife rehabilitators should give serious consideration to their need for pre-exposure vaccination and periodic serologic testing.

People who work closely with wildlife in areas where rabies is prevalent are classified by the ACIP as being in the frequent risk category for potential exposure to virus. All wildlife rehabilitators who work with mammals—and especially those who work with rabies-vector species—should receive a pre-exposure vaccine series and consider the need for periodic serology based upon the prevalence of wildlife rabies in their area.

Because of the added potential for human exposure when handling rabies-vector species, many health departments have

⁵This has engendered fears among some wildlife rehabilitators that an underground traffic of rabies-vector species would result.

expressed concern at attempts to rehabilitate reservoir species. These concerns may be especially amplified during the course of a wildlife epizootic. Citing threat of increased human exposure, some health departments have even successfully lobbied appropriate state agencies to enact legislation forbidding (or, at least, significantly curtailing) the rehabilitation of rabies-vector species.⁵ Such legislation has been directed towards both terrestrial species as well as bats. Implementation of restrictive legislation varies widely from state to state, depending upon the nature and extent of the rabies problem present and upon subjective perceptions of the risks involved. Some states have issued conditional licenses, limiting the rehabilitation of rabies-vector species to those individuals who have received additional specialized training, attend ongoing continuing education seminars, received a pre-exposure vaccine series, and made appropriate modifications to their animal-care facilities.

Although all wilderness travelers should exercise caution in interacting with wildlife, this may be of particular concern to cavers. Because their underground travels frequently bring them into close association—and sometimes even direct physical contact—with bats (Figures 9 and 10), members of the caving community should note that public health authorities consider cavers to be at a relatively higher risk than the general public of sustaining an exposure to virus, including the possibility of an unrecognized exposure.

Cavers familiar with public health recommendations regarding rabies typically base their decision on whether or not to obtain pre-exposure vaccination on a variety of factors. Certainly, serious consideration is given to recommendations of public health authorities. In large measure, however, this is often tempered by individual experience and judgment, taking into account such factors as the expense of vaccination, the nature of one's interaction with bats, the prevalence of rabies infection in bat populations, the perceived likelihood of sustaining an exposure, and, indeed, the entire spectrum of one's caving activity.



Figure 10 - How often do events such as this occur among cavers and how likely is such an occurrence to be associated with an inapparent rabies exposure (i.e., an unrecognized bite or scratch)? In view of the high case fatality rate of clinical rabies, cavers should give serious consideration to their need for pre-exposure vaccination and periodic serologic testing. In the case of an unrecognized exposure to rabies virus, pre-exposure vaccination alone may be lifesaving. Courtesy of Dean Snyder. Greater Allentown Grotto.

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Collectively, such reflection helps to provide cavers with a subjective assessment of their own risk of exposure to rabies virus while underground and serves as the primary basis for making decisions regarding individual need for pre-exposure vaccination. Nevertheless, however low the likelihood of sustaining an exposure to virus—and especially an unrecognized exposure—while caving may be, this must also be carefully balanced against the virtually certain outcome of clinical disease.

In making decisions regarding one's need for pre-exposure vaccination, cavers should not be blinded by an irrational fear that failure to comply with public health recommendations will all but guarantee a fatal exposure to bat-origin rabies virus at some future point in time. Probability of infection remains extraordinarily low. Certainly, there is no reason to develop a bat-related phobia. To be sure, probably no one that fearful of being in relatively close proximity to bats has any really good reason to be underground in the first place. In fact, in spite of the caving community's assignment to a higher risk category of exposure by public health authorities, only two people in this country are known to have died following exposure to rabies while underground. Both deaths have been widely attributed to inhalation of aerosolized virus from the unique environment of heavily populated nursery colonies of Mexican free-tailed bats (*Tadarida brasiliensis*) in the southwestern United States. In actuality, the evidence that inhalation of aerosolized virus was involved in these two human deaths remains questionable.

In terms of sheer number, highly gregarious Mexican free-tailed bats form the largest colonies of any mammal. The inhalant risks for humans entering this type of ecosystem remain poorly defined. It is prudent, however, to consider that unprotected persons entering Mexican free-tail maternity colonies harboring tens of millions of bats may be at increased risk of exposure. Elsewhere, cavers should not be unduly concerned about the possibility of inhalation of viral aerosols; although, it is judicious to avoid confined areas in which enormous populations of bats might be found.⁶ Unfortunately, the number of bats that constitute a "large enough" population cannot be adequately defined. Caves with large bat populations should also be avoided during times of mass movement. Cavers caught in a "bat storm" may be jeopardizing their safety to an unknown degree.

It should also be emphasized that the presence of such incredible numbers of bats in southwestern maternity caves creates a unique environment, markedly different from those systems typically frequented by cavers. As such, the respective sojourns of these two individuals were so far removed from the mainstream of American caving activity that they might arguably be excluded from a practical consideration of caver exposure. Having said this, however, let me also emphasize that cavers should not be lulled into a false sense of security regarding potential risk by the paucity of reported rabies cases among members of the caving community. Cavers continue to encounter bats on a regular basis and not infrequently have direct physical contact with them. In this regard, it is not unusual for bats in flight to have momentary collisions with cavers in narrow passageways

⁶Furthermore, cavers should never disturb either maternity colonies or hibernating populations of bats, since irrevocable damage to the population may be sustained.

and not entirely unknown for bats to occasionally alight on cavers. While such events are not likely to be related to rabies-related disorientation, this can never be assured. And, while cavers exploring temperate-zone caves are generally attired in gloves and a sturdy pair of full-length coveralls, this is not necessarily the case in warmer climes. As such, the risk of sustaining an unrecognized bite or scratch during the course of such an encounter always remains a possibility, however small.

Finally, it is also important to note that there has been at least one published report of a caver in this country who has experienced an apparently unprovoked attack by a confirmed rabid bat while underground. Post-exposure prophylaxis was administered at the time. Certainly, one such report hardly constitutes cause for panic. Unfortunately, there is no information available to suggest how many similar incidents, if any, simply go unreported. There is also no way to fully characterize the nature of physical encounters between bats and cavers, to gauge the relative frequency of such occurrences, or to document what percentage of such encounters may involve infected bats. It is also not known how many cavers actually seek medical attention from year to year following encounters with bats. Clearly, these numbers are relatively low. Nonetheless, these are considerations that should also be taken into account in one's decision-making process regarding individual need for pre-exposure rabies vaccination.

Because no rabies cases other than the two mentioned earlier have been documented in cavers—despite the untold number of man-hours spent underground by members of the National Speleological Society (NSS) every year—and considering the almost complete lack of published information regarding physical encounters between bats and cavers, it is hardly surprising that marginal risk assessments by cavers and recommendations for pre-exposure rabies vaccination by public health authorities are often difficult for members of the caving community to reconcile. According to results of a caver survey conducted by the Centers for Disease Control (CDC) at the NSS 2000 annual convention in Elkins, WV, only 19% of 392 respondents had availed themselves of pre-exposure vaccination. Moreover, fully 14% of respondents (11% of college graduates and 26% of non-graduates) did not consider a bat bite to be of any significance at all in terms of potential exposure to rabies.⁷

While cavers should ideally maintain a clear image in mind as to what kind of encounter with a bat might reasonably be expected to constitute a potential exposure risk (e.g., contact with bare skin and the possibility of a bite or scratch), such assessments may not always be easily made. Apart from such obvious scenarios as a caver receiving a painful bite after foolishly picking up a grounded bat or the highly unlikely case of a painful bite sustained by a caver during an apparently unprovoked "attack" by a clearly disoriented and abnormally behaving bat, it remains difficult at best to evaluate one's actual risk of exposure to rabies following a physical encounter with a bat. Bites and scratches (especially in the

⁷In view of the low number of responses (392 out of 1,508 convention attendees) and the possibility of a selection bias among respondents, survey results may not accurately reflect vaccination status or knowledge of bat rabies among members of the caving community as a whole.

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Figure 11 - The common vampire bat, *Desmodus rotundus*, is a well-recognized reservoir of rabies virus. Courtesy of Randall D. Babb (©). Arizona Game and Fish Department.

absence of bleeding) will be virtually impossible to even see in the darkened conditions of a cave, may be confused with a momentary scrape against a rock, or may not even be noticed at all. And, while sick or grounded bats are much more likely to be rabid, even healthy-appearing bats may be infected and capable of transmitting disease. Certainly, a number of factors come into play, many of which will be almost impossible to adequately assess. In spite of considerable discussion devoted to the subject of bats and rabies by NSS members each year, it is little wonder that many cavers still remain a bit bewildered by it all.

In the final analysis, cavers will either elect to receive a pre-exposure vaccine series should they consider it justified by the potential risk of exposure and concern about the likely outcome of clinical disease or decline vaccination if they consider that the risks don't merit it. The default position of most undecided cavers will likely fall on the side of declining vaccination.

Regardless of whether one decides to obtain a pre-exposure vaccine series or not, it is most important that cavers always give serious consideration to the nature of any physical encounter with a bat...especially if no heavy clothing barrier intervened. In this regard, it is imperative that cavers not succumb to the false sense of security afforded by the small vector hypothesis—the mistaken belief that small animals like bats are of only limited significance as disease vectors—and simply ignore a potential exposure to virus following a physical encounter with a bat. And while not intentionally trying to sound an alarming note, let me reiterate that human deaths from bat-origin rabies virus are not associated with a failure of post-exposure prophylaxis, but with a failure to seek medical attention in the first place (either because an exposure was not recognized or because it was not considered to be of significant concern).

As such, cavers should appreciate the fact that there is nothing to be lost by consulting with public health officials should they have a physical encounter with a bat that might *reasonably* be associated with a potential exposure to virus. Moreover, a bat involved in such an encounter might be carefully collected, if possible to do safely, and submitted for ra-

bies testing. Not only will a negative report obviate the need for unnecessary post-exposure prophylaxis, it will also provide significant peace of mind for the individual involved. Cavers loathe to consider the capture and testing of bats under such circumstances may regard a pre-exposure vaccine series as a more justifiable alternative. Any potential decisions regarding the need for post-exposure prophylaxis can then be made in consultation with public health officials.

Keeping in mind the fact that the probability of exposure to a rabid insectivorous bat does remain extraordinarily low, a special case may exist among those cavers who routinely mount expeditions to Latin America. In recent years, this area has become something of a new frontier of exploration and cavers worldwide have been attracted to it in increasing numbers. As home range of the common vampire bat, *Desmodus rotundus* (Figure 11), this region should be one of particular concern to cavers. Possibly within caves, but particularly in the rustic setting of primitive campsites, cavers remain exposed to vampire bats and this may invite attack.

The habit of these bats in seeking blood meals is of significance in that they are almost ideal vectors of rabies. Attacks on humans by vampire bats are not uncommon and hundreds of people are believed to have died of vampire-transmitted rabies. Cavers exposed to potential attacks by these well-established reservoirs of rabies virus may be placing themselves at increased risk. In this regard, persons embarking on extended expeditions to this region of the world should take special precautions to ensure safety.

In light of the documented presence of rabies and a variety of rabies-related viruses⁸ in bat populations worldwide, all people who routinely handle bats—regardless of their geographic location—should consider themselves at increased risk of potential exposure, receive a pre-exposure vaccine series, and have their titers periodically assessed. Certainly, for bat biologists (whose work may entail handling bats) and bat rehabilitators (who minister to sick, injured, and orphaned bats) working anywhere in the world, pre-exposure vaccination is an imperative.⁹

The prevalence of rabies and rabies-related viruses in bat populations varies widely from species to species, as well as geographically. While several species of bats are known to be reservoirs of rabies virus, others are suspected and a bewildering array of viral variants is known to circulate among bats. Our understanding of bat rabies is hampered by the relative rarity of even encountering, let alone sampling, many species. Because the epidemiology of rabies in insectivorous bats is complex and far from understood, distinction between species is not made insofar as post-exposure prophylaxis is concerned. Thus, from the public health perspective, all bats are considered to be rabies-vector species and anyone—whether wildlife rehabilitator,

⁸Classical rabies virus - the Americas; Lagos bat virus, Duvenhage, and perhaps Mokola virus - Africa; European bat lyssavirus types 1 and 2 - Europe; and Australian bat lyssavirus - Australia.

⁹Conventional rabies prophylaxis has not been shown to be effective in animal models against several recently discovered rabies-related lyssaviruses known to circulate among various Eurasian bat populations: Aravan, Khujand, Irkut, and West Caucasian bat viruses. The public health significance of these novel viruses remains to be fully assessed.

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Rabies postexposure prophylaxis schedule — United States, 2008

Vaccination status	Treatment	Regimen*
Not previously vaccinated	Wound cleansing	All postexposure prophylaxis should begin with immediate thorough cleansing of all wounds with soap and water. If available, a virucidal agent such as povidine-iodine solution should be used to irrigate the wounds.
	Rabies immune globulin (RIG)	Administer 20 IU/kg body weight. If anatomically feasible, the full dose should be infiltrated around the wound(s) and any remaining volume should be administered intramuscularly (IM) at an anatomical site distant from vaccine administration. Also, RIG should not be administered in the same syringe as vaccine. Because RIG might partially suppress active production of antibody, no more than the recommended dose should be given.
	Vaccine	Human diploid cell vaccine (HDCV) or purified chick embryo cell vaccine (PCECV) 1.0 mL, IM (deltoid area [§]), one each on days 0 [¶] , 3, 7, 14, and 28.
Previously vaccinated [†]	Wound cleansing	All postexposure prophylaxis should begin with immediate thorough cleansing of all wounds with soap and water. If available, a virucidal agent such as povidine-iodine solution should be used to irrigate the wounds.
	RIG	RIG should not be administered.
	Vaccine	HDCV or PCECV 1.0 mL, IM (deltoid area [§]), one each on days 0 [¶] and 3.

* These regimens are applicable for all age groups, including children.

[†] Any person with a history of a complete pre-exposure or postexposure vaccination regimen with HDCV, PCECV, or rabies vaccine adsorbed, or previous vaccination with any other type of rabies vaccine and a documented history of antibody response to the prior vaccination.

[§] The deltoid area is the only acceptable site of vaccination for adults and older children. For younger children, the outer aspect of the thigh can be used. Vaccine should never be administered in the gluteal area.

[¶] Day 0 is the day the first dose of vaccine is administered.

Table 2 - Recommendations for post-exposure rabies prophylaxis in the United States. Reproduced with permission from the *Recommendations of the Advisory Committee on Immunization Practices, Human Rabies Prevention – United States* (2008).

caver, bat biologist, or member of the general public—who sustains a possible bite or scratch from any species of bat should be managed accordingly.

Post-Exposure Rabies Prophylaxis

Post-exposure rabies prophylaxis involves the administration of both human rabies immune globulin (HRIG) and rabies vaccine (Table 2). Vaccine contains inactivated rabies antigen, which stimulates the body's immune system to produce its own rabies-neutralizing antibodies (active immunization). Rabies immune globulin, on the other hand, contains pre-formed antibodies, which will provide protection (passive immunization) until the body begins to mount a suitable immune response of its own.

Of course, it remains important for all people who have received pre-exposure vaccination against rabies to understand exactly what this means in practical terms. Of most significance, in this respect, is the fact that pre-exposure vaccination simplifies, but does not completely eliminate the need for post-exposure prophylaxis in the event of a possible or confirmed exposure to rabies virus. In particular, it reduces the number of doses of vaccine required and eliminates the requirement for HRIG.

In this country, standard post-exposure prophylaxis in an individual who has not been previously vaccinated against rabies includes both passive and active immunization. Active immunization consists of a five-dose vaccine series, begun as soon as possible after exposure. Vaccine is administered as a single intramuscular injection on what is defined as day 0 and followed by an additional dose on days 3, 7, 14, and 28. Passive immunization with HRIG should be administered at the same time that the first dose of vaccine is given, but can still be given up to seven days after the first injection. Specific guidelines for the administration of anti-rabies pharmaceuticals should be

closely adhered to by medical personnel to ensure efficacy of post-exposure management. Public health officials can be consulted for specific concerns regarding management of complicated cases, such as administration of HRIG in cases involving multiple bite wounds, management of ongoing prophylaxis in individuals who have begun treatment in developing nations with pharmaceuticals of questionable efficacy, management of persons who have suffered repeated exposures, etc.

On the other hand, for those who have received pre-exposure vaccination against rabies with a modern cell-culture vaccine, have previously received a full course of post-exposure prophylaxis with a potent cell-culture vaccine, or have been immunized with other vaccines and have had a documented titer of rabies-neutralizing antibodies, post-exposure prophylaxis consists of two booster doses of vaccine only—one dose administered immediately and a second dose given three days later. HRIG should not be administered, since its concurrent use with vaccine can suppress antibody production in pre-exposure-immunized individuals.

Regardless of one's vaccination status, the most important first step in the management of a bite or scratch wound is thorough cleansing of the site with soap and water and a mild virucidal agent, such as a dilute povidone-iodine solution. Any known or suspected exposure to rabies virus should then be followed by prompt evaluation by a knowledgeable medical professional regarding regional concerns in rabies epidemiology, disposition of the animal involved (e.g., availability for testing or quarantine, if the latter is appropriate—note: quarantine does not apply to bats), and the potential need for post-exposure prophylaxis. Even if rabies is not a concern, general wound management may still warrant medical attention since any number of serious complications may attend an animal bite or scratch.

While the administration of post-exposure prophylaxis is generally not considered to be a medical emergency in this country, it is a medical urgency. Hence, appropriate decisions

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regarding the need for proper management should be made in a timely fashion and without undue delay. Decision-making with regard to the need for administration of post-exposure prophylaxis should be made by qualified medical personnel and based on a careful assessment of a number of factors. Because the complexity of such decision-making processes may vary considerably from one case to another, medical practitioners should not hesitate to avail themselves of expert consultation with local and state public health officials or with rabies experts at the CDC, if needed.

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*On February 15, 2009, ProMED (International Society for Infectious Diseases) provided an update on the status of this patient. According to Dr. Gustavo Trindade Henriques Filho, the patient's attending physician, the boy had been discharged from the critical care unit of the Oswaldo Cruz University Hospital (Pernambuco State University, Brazil) on February 4, 2009. At this time, he is conscious and speaking. Although his cognitive function is good, "motor limitations" [the nature and severity of which remain unspecified at present] still exist.

RABIES SEROLOGY AND THE RFFIT

Determination of a Rabies Titer and What it Really Means

Danny A. Brass

Human rabies is an uncommon condition in the United States, averaging less than three cases annually. This is largely due to eradication of the canine variant of rabies virus and to the availability of efficacious post-exposure prophylaxis. Because many people still face a higher-than-average or somewhat unpredictable risk of exposure to rabies virus, a pre-exposure vaccination series is also an important safeguard for various individuals.

The Concept of a Rabies Titer

As a natural consequence of having received a pre-exposure vaccine series against rabies, people who are members of one or more of the groups generally recognized as being at a higher-than-average risk of exposure are often interested in the testing process by which one's immune response to vaccine administration is determined. In particular, interest tends to focus around the concept of the *rabies titer*: how it is determined, how it is interpreted, and what it really means in terms of protection against development of clinical disease following exposure to this virus. The testing procedure itself is highly complex and the results are frequently misunderstood.

The rabies titer is a functional measure of one's immune response to vaccination against rabies virus. In actuality, it is a measure of the amount of *rabies-neutralizing antibodies* present in a person's blood. Compared to other aspects of the body's immune response that play important roles in protection against rabies—notably the development of immunologic memory and the complex features of cell-mediated immunity—antibody concentration is the easiest to document and quantitate. Moreover, the production of rabies-neutralizing antibodies is known to be the most critical factor in preventing rabies. As such, it serves as the best available proxy for the level of protection one has against development of clinical disease following exposure to this lethal virus. However, for reasons discussed below (see the section on *What is Actually Meant by an Adequate Response to Vaccination?*), it should be noted that the rabies titer is really not a true measure of one's level of protection against clinical rabies. Nevertheless, it is the best substitute for that measure currently available.

Parameters of the RFFIT as a Viral Neutralization Test

At present, the gold standard for determining a rabies titer is the *Rapid Fluorescent Focus Inhibition Test* or RFFIT. In fact, it is the only test recommended by the Centers for Disease Control and Prevention (CDC) and the Advisory Committee on Immunization Practices (ACIP) for this purpose. Determination of a rabies titer by RFFIT is available to anyone who has been vaccinated against rabies; however, it tends to be of most value for those who routinely face a relatively high risk of exposure.

The RFFIT is based on an indirect determination of the concentration of rabies-neutralizing antibodies in a blood sample. The very presence of such antibodies in an individual's

Abbreviations used in this article

ACIP	Advisory Committee on Immunization Practices
CDC	Centers for Disease Control and Prevention
ELISA	enzyme-linked immunosorbent assay
IU	International Unit
mL	milliliter
RFFIT	rapid fluorescent focus inhibition test
TCID₅₀	tissue culture infectious dose ₅₀
WHO	World Health Organization

blood is an indication that the immune system has responded to the vaccine (note: this would be the case regardless of whether the vaccine had been administered as either a pre- or post-exposure series).

In practice, the RFFIT measures how much of a known quantity of rabies virus is neutralized (i.e., inactivated) by antibodies present in a small sample of an individual's blood. The result, most commonly reported in this country as the rabies titer, is expressed as a dilution (e.g., 1:5) of the original blood sample. This is a dimensionless number (i.e., one without units). By comparing the measured titer to known reference standards, the RFFIT can also be used to determine an actual concentration of rabies-neutralizing antibodies in the blood. The unit of measurement of antibody concentration is the International Unit (IU) and the concentration of rabies-neutralizing antibodies in a serum sample is expressed as International Units per milliliter (IU/mL).

In simplified form, the standard RFFIT assay works like this: A measured amount of blood serum¹ is mixed with a specified quantity of rabies virus. If neutralizing antibodies to the rabies virus are present in the serum sample, some (or all) of the rabies virus will be neutralized. Once neutralized, the virus is no longer capable of infecting cells. After a suitable period of incubation, a suspension of cells that are known to be vulnerable to rabies infection (e.g., baby hamster kidney cells or mouse neuroblastoma cells) is then added to the serum/virus mixture. If any intact rabies virus is still present in the mixture, it will infect the cells. Following another period of incubation, the cells are then observed under a microscope for evidence of infection with virus.

The presence of virus within cells is detected by staining them with a specially prepared anti-rabies antibody to which a fluorescent-label has been attached. The antibody will bind to

¹Whole blood consists of a liquid portion, called plasma, and formed elements or blood cells. The latter include red blood cells (or erythrocytes), white blood cells (or leukocytes), and platelets (or thrombocytes). A sample of whole blood quickly becomes a somewhat gelatinous mass, called a clot. Following clot formation, a small amount of fluid, called serum, oozes from the clotted blood. Serum is similar to plasma; however, the clotting factors are no longer present, having been used up in formation of the clot. Serum is the fraction of blood containing antibodies. Determining the presence and amount of specific immune substances, such as antibodies, in serum is the discipline of serology.

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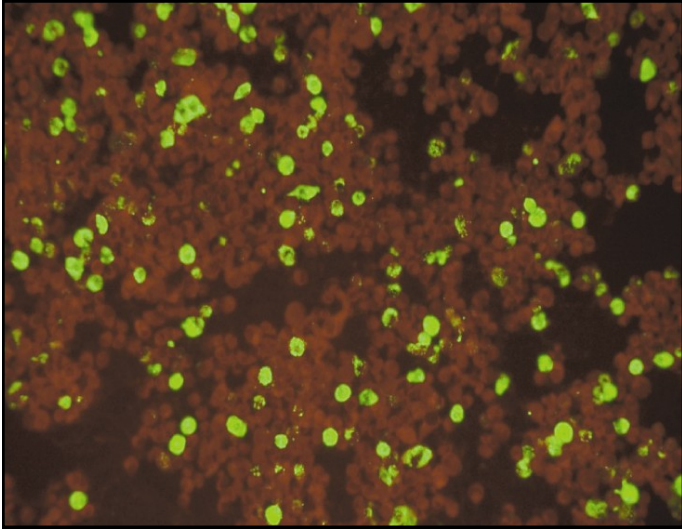


Figure 1 - Identifying the presence of rabies virus based on fluorescence microscopy. The apple-green fluorescence is a marker for infected cells. Magnification x160. Courtesy of Susan Moore. Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University.

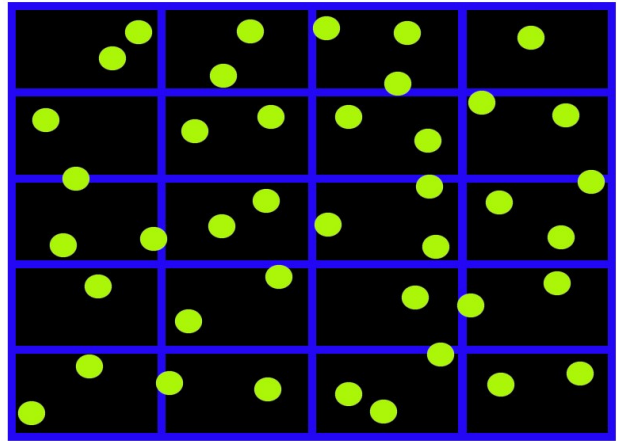
any rabies virus present and the characteristic apple-green color of the fluorescent label—visible as it fluoresces when observed under a fluorescence microscope—will document the presence of rabies virus within infected cells (Figure 1).

The number of cells infected with virus will be directly related to the number of intact virus particles that had been present in the mixture. In other words, the larger the amount of intact virus present, the more cells will be infected. If the serum sample being tested contains no neutralizing antibodies to the rabies virus, all of the cells in the test system will be infected. On the other hand, if the serum does contain rabies-neutralizing antibodies, varying amounts of virus will be inactivated and fewer cells will be infected. If the concentration of antibodies in the serum sample is high enough to completely inactivate all of the test virus (i.e., 100% viral neutralization), there will be no intact virus left and no infected cells will be observed at this stage. In making this determination, 20 cellular fields are observed under the microscope and the number of fields containing infected cells is recorded (Figure 2).

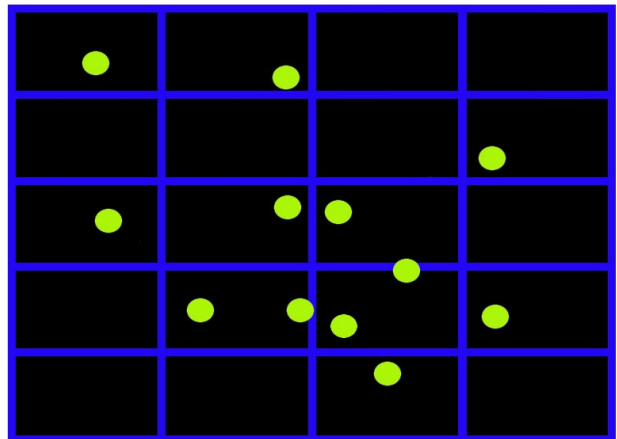
In actual application, the original serum sample to be tested is first diluted. In fact, a series of dilutions is typically made (Figure 3). In the United States, the RFFIT is traditionally based on a fivefold dilution series (1:5, 1:25, 1:125, 1:625, and so on); however, some laboratories may make use of a twofold or even threefold dilution series. Thus, the serum samples tested by the RFFIT will be a range of dilutions of the original blood specimen submitted for testing. Each dilution will be tested individually. As the original serum sample is serially diluted, the concentration of rabies-neutralizing antibodies, if any are present, will be proportionally decreased.

Depending on the quantity of rabies-neutralizing antibody present in the original (i.e., undiluted) sample, successive dilutions may or may not retain enough antibodies to achieve 100% viral neutralization. If the concentration of rabies-neutralizing antibodies in the original blood sample is very high, neutralization of a carefully specified amount of test

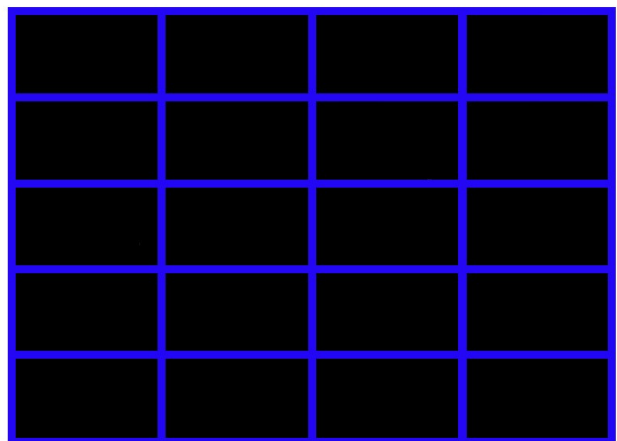
virus can still be achieved at successively higher and higher serum dilutions. The basic idea behind the RFFIT is to keep diluting the serum in order to see just how much the original



20/20 fields with infected cells



10/20 fields with infected cells



0/20 fields with infected cells

Figure 2 - Counting and recording the number of fields in which infected cells are observed.

RABIES SEROLOGY AND THE RFFIT

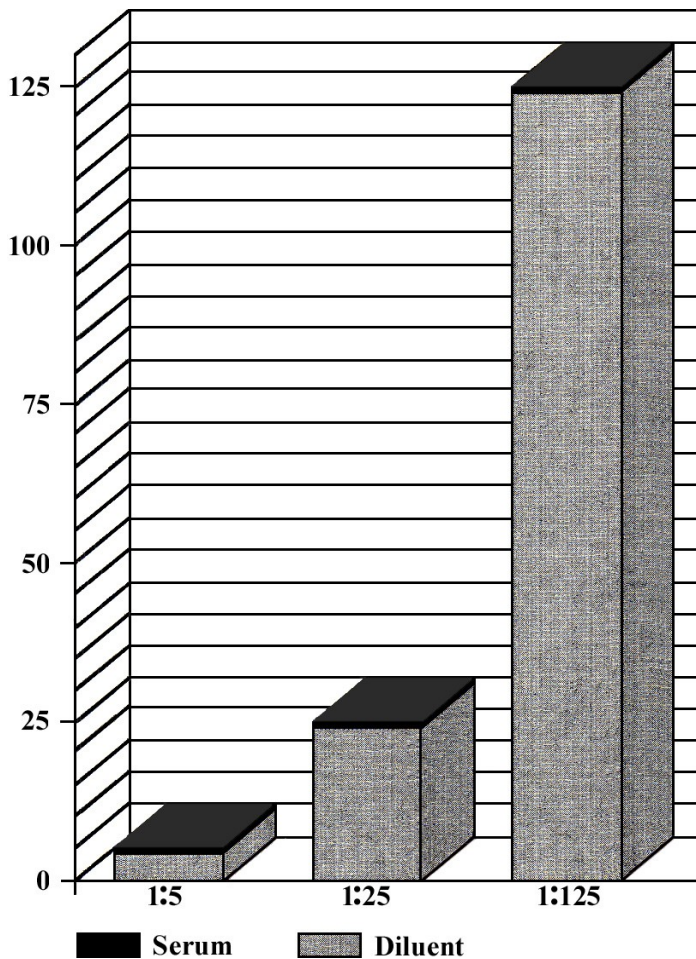


Figure 3 - The RFFIT is traditionally based on a fivefold dilution series, a portion of which is illustrated here. The larger the second number in the expression, the more dilute the sample is. Thus, a 1:125 sample is more dilute than a 1:25 sample, which is more dilute than a 1:5 sample.

sample can be diluted and still contain a high enough concentration of rabies-neutralizing antibodies to inactivate a standard amount of virus in a carefully prepared test sample. The more a sample can be diluted and still retain enough rabies-neutralizing antibody activity to inactivate a specified amount of virus, the higher the concentration of antibody in the original serum specimen.

In practice, most laboratories base their evaluation of a serum sample on its capacity to neutralize enough virus so that 50% (10/20) of the 20 observed fields contain infected cells (note: some laboratories base their evaluation of serum samples on their capacity to neutralize 100% of the test virus, so that no infected cells are observed). The 50% endpoint determination is used because it is easy to visualize increases or decreases from this value. One should note that an endpoint determination of 50% infected fields is not equivalent to neutralization of 50% of virus in the test system. In fact, because of the way the test procedure is set up—most notably by the addition of a relatively large amount of test virus—this finding would actually be equivalent to between 97 to 99% viral neutralization, the exact percentage depending on the amount of virus used in the test.²

In this country, results of the RFFIT are primarily expressed as the rabies titer. The rabies titer, as specified by parameters of the test, is defined as that serum dilution at which 50% of the observed fields in the test system contain infected cells. Accordingly, observation of 10/20 infected fields in the 1:5 dilution sample specifies a titer of 1:5; observation of 10/20 infected fields in the 1:25 dilution sample specifies a titer of 1:25; observation of 10/20 infected fields in the 1:125 dilution sample specifies a titer of 1:125; and so on. This is illustrated in the column associated with 10/20 infected fields in Table 1, which expresses these titers as reciprocals: 5, 25, 125, etc. Because the same amount of virus is used to test each dilution, a sample with a measured titer of 1:125, for example, comes from a serum sample that originally contained more rabies-neutralizing antibodies than one from a sample with a measured titer of 1:25 and so on.

At this juncture, a very important point regarding the 1:5 titer should be made. Although this titer can technically be defined as described above, a different meaning is actually assigned to it in clinical practice. As such, the 1:5 titer represents a special case and I will come back to this issue further on.

The Reed-Muench Calculations

Based on the parameters of the RFFIT described above, making a titer determination of 1:5, 1:25, 1:125, 1:625, etc. on any given serum sample is a fairly straightforward process. These would simply correspond to those dilutions in which half of the observed fields contained infected cells (i.e., an endpoint determination of 50%). But how would a titer based on any of the standard test dilutions be determined if the number of observed fields containing infected cells was not 10/20, but rather 2/20 or 6/20 or 17/20?

In these cases, the dilution of a particular sample that would have produced a 50% endpoint measurement can be calculated by a mathematical formulation known as the *Reed-Muench method*. By making use of these computations, a calculated 50% endpoint determination can be derived for any dilution of a given serum sample, no matter how many fields are observed to contain infected cells.

The results of these calculations are evident in Table 1, which is essentially a chart of the calculated 50% endpoint values. All of the titer determinations in the 10/20 column were made in direct association with the fact that half of the observed fields at any given dilution contained infected cells. That the measured titers of such samples are equivalent to the dilution used is a specified parameter of the test system. However, every other titer on the chart is an extrapolated value,

²One tissue culture ID₅₀ (TCID₅₀) is the infectious dose of virus that will result in 50% of the observed fields having one or more infected cells. Between 30 and 100 TCID₅₀ are added to each serum dilution, a quantity of virus that is obviously enough to infect all of the cells in all of the fields many times over. If only 50% of the observed fields from any given sample contain infected cells, an amount of virus equal to only one TCID₅₀ survived intact; the remaining virus (between 29 and 99 TCID₅₀) had all been inactivated by interaction with the rabies-neutralizing antibody present in the serum. Thus, 29/30 (97%) to 99/100 (99%) of the test virus had been neutralized.

RABIES SEROLOGY AND THE RFFIT

	Number of Infected Fields	0/20	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	1:5	0.110	0.110	0.1	0.10	0.09	0.09	0.08	0.07	0.07	0.06	0.05										
	Titer	11	11	10	10	9	9	8	7	7	6	5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5
1	1:5	0.560	0.540	0.510	0.500	0.450	0.430	0.400	0.360	0.330	0.290	0.250	0.220	0.190	0.170	0.160	0.150	0.140	0.130	0.120	0.120	
	1:25																					
	Titer	56	54	51	50	45	43	40	36	33	29	25	22	19	17	16	15	14	13	12	12	
2	1:25	2.8	2.7	2.6	2.5	2.3	2.2	2.0	1.8	1.6	1.5	1.3	1.1	1.0	0.9	0.8	0.8	0.7	0.7	0.6	0.6	
	1:125																					
	Titer	280	270	260	245	230	215	200	180	163	145	125	110	95	85	80	75	70	65	60	60	
3	1:25	14.0	13.0	13.0	12.0	11.0	11.0	10.0	9.0	8.0	7.0	6.3	5.4	4.8	4.3	4.0	3.7	3.4	3.2	3.0	2.9	
	1:625																					
	Titer	1400	1300	1300	1200	1100	1100	1000	900	800	700	625	540	480	430	400	370	340	320	300	290	
4	1:25	70.0	67.0	64.0	60.0	57.0	53.0	49.0	45.0	41.0	36.0	31.3	27.0	24.0	22.0	20.0	18.0	17.0	16.0	15.0	15.0	
	1:3125																					
	Titer	7000	6700	6400	6000	5700	5300	4900	4500	4100	3600	3125	2700	2400	2200	2000	1800	1700	1600	1500	1500	
5	1:25	349.0	335.0	320.0	300.0	286.0	267.0	248.0	226.0	204.0	181.0	156.3	135.0	120.0	108.0	99.0	91.0	85.0	81.0	76.0	73.0	
	1:15625																					
	Titer	34900	33500	32000	30000	28600	26700	24800	22600	20400	18100	15625	13500	12000	10800	9900	9100	8500	8100	7600	7300	
6	1:25	1747.0	1675.0	1598.0	1516.0	1429.0	1336.0	1237.0	1133.0	1022.0	904.0	781.3	675.0	597.0	539.0	493.0	457.0	427.0	403.0	382.0	365.0	
	1:78125																					
	Titer	174700	167500	159800	151600	142900	133600	123700	113300	102200	90400	78125	67500	59700	53900	49300	45700	42700	40300	38200	36500	

Table 1 - One of many charts showing the relationship between serum dilutions, number of observed fields containing infected cells, titer determinations, and rabies-neutralizing antibody concentrations. The number of infected fields are determined by direct observation, antibody concentrations (in IU/mL) are based on comparisons with known reference standards, and titer values are largely extrapolated from the Reed-Muench calculations. Courtesy of Susan Moore.

mathematically derived from the Reed-Muench calculations and based on the observed number of fields containing infected cells. They represent the dilution of any given serum sample—and, hence, the calculated rabies titer—that *would have* resulted in a 50% endpoint determination (i.e., the observation of 10/20 fields containing infected cells). Thus, if a 1:625 dilution of a serum sample gives a test result of 14/20 infected fields, the calculated rabies titer for that specimen would be 1:400. Put another way, a 1:400 dilution of the original serum sample would have given a test result of 10/20 infected fields.

For laboratories that base all of their titer determinations on complete neutralization of virus as an endpoint, the titer is more simply defined as the last dilution at which no infected cells are observed. Hence, no such mathematical extrapolation is required.

Evaluating and Interpreting RFFIT Results

In the United States, guidelines established by the ACIP define the minimum acceptable antibody level that documents a suitable immune response to rabies vaccination as one that achieves complete neutralization of virus at a 1:5 serum dilution. Put more simply, this means that if a fivefold dilution of an individual's blood serum (i.e., 1 part serum in 4 parts diluent) still contains enough antibodies to neutralize 100% of a carefully specified amount of rabies test virus, that person is considered to have adequately responded to vaccine administration. For this reason, the initial goal of rabies immunization is to develop a high enough concentration of rabies-neutralizing antibodies in a person's blood so that complete neutralization of virus—as determined by parameters

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	0 / 20	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1:5	0.1	0.1	0.1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Titer	11	11	10	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5	<1:5

Table 2 - One of many charts showing the way RFFIT results are interpreted. Only information pertaining to the 1:5 dilution is presented. Those samples in which complete (or almost complete) neutralization of virus could not be observed at this dilution are designated as having a rabies titer of < 1:5. Courtesy of Susan Moore.

specified by the RFFIT—is achieved at a 1:5 (or greater) serum dilution.

A look across the 1:5 dilution row of Table 1 (top panel) shows that the titer corresponding to complete neutralization of test virus (i.e., the observation of 0/20 infected fields) at that dilution is actually 1:11. Thus, in accordance with the parameters used to set up the test system, a 1:11 titer represents the minimally acceptable criterion (as defined above) for considering that an adequate response to rabies vaccination has occurred. However, because of inherent variability in biological test systems such as this, some laboratories will accept the 1:10 titer (i.e., almost complete neutralization) as well. Use of the 100% endpoint (0/20 infected fields) as opposed to a 50% endpoint (10/20 infected fields) provides a safety factor that has been retained in evaluation of serum samples at the 1:5 dilution (the reason for retention of this safety factor is discussed in the section on *The World Health Organization Guidelines*).

Based on the definition of a rabies titer given previously (and in accordance with the parameters of the RFFIT test system and the Reed-Muench calculations), if 0/20 infected fields are observed in the 1:5 dilution sample, the calculated rabies titer of this specimen would be at least 1:11 (note: don't confuse the definition of a rabies titer with the serum dilution being evaluated). Two pieces of information come out of the association of a 1:11 titer with the observation of 0/20 infected fields in this sample: 1) a fivefold dilution of the original serum sample from which this specimen had been obtained will neutralize approximately 100% of the virus used in the RFFIT test system and produce an observed result of 0/20 (or 1/20) infected fields, and 2) a 1:11 dilution (1 part serum in 10 parts diluent) of the original serum sample from which this specimen had been obtained will neutralize approximately 97 to 99% of the virus used in the RFFIT test system and produce an observed result of 10/20 infected fields.

And now, we come to the confusing part of RFFIT interpretation. As indicated above, the 1:11 rabies titer is the titer associated with complete viral neutralization at the 1:5 dilution (i.e., the titer that corresponds to the ACIP's minimally acceptable standard for an adequate response to rabies vaccination). However, most people who have received a rabies vaccine series are familiar with the generally recognized criterion that a 1:5 titer—not a 1:11 titer—represents the cutoff value for separating an acceptable from an unacceptable response to vaccine administration. So, what is the reason for this apparent discrepancy? Actually, this is due to a strictly defined relationship that has been assigned to the 1:11 and 1:5 titers.

In order to avoid burdening the general public with excessive details about titers and dilutions, a decision had long ago

been made that the cutoff value for what was considered to be an adequate response to vaccine administration (i.e., the complete neutralization of test virus at the 1:5 dilution) would itself define the limits of what would be called a 1:5 titer. This designation has remained firmly entrenched in more generalized serology literature. In this regard, note the subtle differences in wording between 1) serology recommendations meant for more general consumption (*a rabies titer of \geq 1:5 is evidence of an adequate response to rabies vaccination*), and 2) those of the ACIP meant for medical professionals (*the minimum acceptable antibody level that documents a suitable immune response to rabies vaccination is one that achieves complete neutralization of virus at a 1:5 serum dilution*).

We can make sense of these two recommendations by once again looking at the 1:5 dilution row of Table 1. Beneath the heading of 10/20 infected fields, we can see the associated titer of 1:5 (simply expressed as the reciprocal: 5). Titers associated with samples resulting in more than 10 infected fields are simply designated as being < 1:5. Titers associated with samples resulting in less than 10 infected fields are > 1:5 and increase in association with decreasing numbers of observed infected fields. At the far left side of the row are the test results corresponding to complete or near-complete neutralization of virus. According to the Reed-Muench calculations, these are associated with titers of 1:11 and 1:10, respectively. At this dilution, only those test results that fall within this latter range are considered to meet the minimum ACIP requirements of an adequate response to vaccination (i.e., complete neutralization of virus at the 1:5 dilution).

Now compare this information to that presented in Table 2, which explains how test results at the 1:5 dilution are actually interpreted in clinical practice. For the purpose of evaluating test results at this dilution, the operational definition of a 1:5 titer is slightly different than that described earlier and as represented in Table 1. Accordingly, it is not the observation of 10/20 infected fields at this dilution that defines what is commonly known as a 1:5 titer. Rather, it is the cutoff point for complete (or almost complete) viral neutralization—the portion of the chart that correspond to a titer of 1:10 and 1:11—that defines the limits of a 1:5 titer. Hence, the operational definition of a 1:5 titer is one that corresponds to complete viral neutralization at the 1:5 dilution. Anything less than this (including titers of 1:6, 1:7, 1:8, and 1:9 shown in Table 1) is defined as being a < 1:5 titer and anything greater than this (i.e., moving now into the 1:25 dilution row) is defined as a > 1:5 titer.

In light of this revised definition of a 1:5 titer, we can now understand the meaning of the ACIP recommendations in terms of titers. Thus, based on work carried out by researchers at the CDC, the ACIP considers a titer of 1:5 to be the mini-

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**RAPID FLUORESCENT FOCUS INHIBITION TEST
DEPARTMENT OF VETERINARY DIAGNOSIS
KSU VETERINARY MEDICAL CENTER
1800 DENISON AVENUE
MANHATTAN KS 66506-5600
(785) 532-4483**

RECEIVED DATE: June 03, 2008

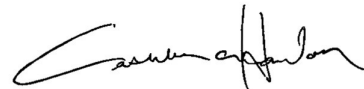
REPORT DATE: June 16, 2008

SUBMITTED BY

DANNY BRASS

RFFIT #	PATIENT	DRAW DATE	RABIES TITER-RESPONSE
RF8- 6831-1	DANNY BRASS	6/2/2008	1:400

**RABIES NEUTRALIZING ANTIBODY TITRATION
(RFFIT) RESULT VERIFIED BY:**



**Dr. Cathleen A. Hanlon, Director
Rabies Laboratory KSVDL**

Results are confidential, personal health information. All disclosures must be in accordance with HIPAA.

*** READ > AS "GREATER THAN" ** IN HUMANS, A TITER OF 1:5 OR GREATER IS CONSIDERED ACCEPTABLE AS PER ACIP**

Figure 4 - Sample report of a quantitative endpoint RFFIT determination from the Kansas State University Diagnostic Laboratory. This titer corresponded to a rabies-neutralizing antibody concentration of 4.9 IU/mL.

mally acceptable value that documents an adequate response to rabies vaccination, where the meaning of a 1:5 rabies titer is as described above. Accordingly, titer results are often just reported as $\geq 1:5$ (i.e., an adequate response to vaccination) or $< 1:5$ (i.e., an inadequate response to vaccination). This is how results of qualitative screening determinations would be reported. However, some people may prefer to see an actual quantitative endpoint measurement (Figure 4). The latter is a study in which the full complement of serum dilutions beyond the 1:5 level are made in order to establish the highest dilution at which a 50% endpoint determination (i.e., observing 10/20 fields with infected cells) can still be seen (note: for dilutions beyond the 1:5 level, results are based on a 50% endpoint determination as opposed to the 100% endpoint determination used for the 1:5 dilution).

Titer determinations have special significance for people considered to be at a higher-than-average risk of being exposed to rabies virus.

What is Actually Meant by an Adequate Response to Vaccination?

At this point, it is important to provide some perspective as to what the concept of an adequate response to rabies vaccination (i.e., 100% viral neutralization at a 1:5 serum dilution or a so-called "1:5 rabies titer") actually means. As mentioned previously, RFFIT determination of a rabies titer in the United

States has traditionally been based on a standard fivefold dilution series. The first serum dilution in this series, of course, is the 1:5 dilution. A laboratory determination of 100% viral neutralization at this dilution documents the presence of rabies-neutralizing antibodies in a person's blood serum, unequivocally establishing that he or she has satisfactorily responded to the rabies vaccine.

The development of immunologic memory, also called an *anamnesic response*, is a critical feature of the immunization process. In the case of rabies virus, for example, certain cells in the immune system (appropriately referred to as memory cells) retain a "memory" of their exposure to the inactivated virus in the vaccine. Thus, they remain primed for antibody production should it become necessary. Upon encountering either virulent rabies virus or a booster dose of vaccine at some future point in time, these cells will quickly begin producing rabies-neutralizing antibodies. This rapid shift of antibody production into high gear is one of the mainstays of the body's defense against rabies infection; although, it should be noted that a variety of other, poorly understood factors also play important roles in the protection against development of clinical disease. An immune response sufficient to achieve complete viral neutralization at the 1:5 dilution is known to be associated with development of a population of memory cells; hence, the significance of the 1:5 dilution and the justification for the ACIP's recommendation that an antibody titer of $\geq 1:5$ demonstrates an adequate immune response in persons who

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have received a pre-exposure vaccine series against rabies.

A significant source of confusion for many people concerns the relationship of an adequate rabies titer and the concept of a protective level of antibody in the bloodstream. Even a rabies titer of $\geq 1:5$ cannot necessarily be equated with an absolute level of protection. In point of fact, there is no definitively established titer or antibody concentration in humans that unequivocally guarantees "protection" against infection with rabies virus. Rather, the 1:5 rabies titer merely confirms that the body's immune system has responded to administration of the vaccine by production of rabies-neutralizing antibodies and a cache of memory cells...the principal goals of immunization. Consequently, it is imperative that individuals who have been previously vaccinated against rabies appreciate the fact that an abbreviated post-exposure regimen is still required following a potential exposure to virus.

In order to conclusively establish that any particular titer or serum antibody concentration actually confers protection against the development of clinical disease, a series of human vaccine trials would have to be carried out in which human subjects, with varying levels of rabies-neutralizing antibodies, would be challenged with live, virulent rabies virus. Results would then be recorded as the survival or death of test subjects. Clearly, carrying out such trials would be highly unethical.

The World Health Organization Guidelines

Another common source of confusion derives from the fact that the World Health Organization (WHO) Expert Committee on Rabies makes slightly different recommendations than the ACIP does in this country. Thus, the WHO definition of an adequate response to rabies vaccination is not based on a rabies titer (i.e., a specific serum dilution) per se, but rather on a measured rabies-neutralizing antibody concentration of at least 0.5 IU/mL of serum. The latter is calculated from a RFFIT determination by comparing the amount of antibody present in a serum sample with that of appropriate reference sera containing known amounts of rabies-neutralizing antibody.

The reasons behind this difference in the WHO recommendations are related to issues of non-specific neutralization of rabies virus in the test system (i.e., the inactivation of rabies virus due to factors other than the presence of rabies-neutralizing antibodies). For various technical reasons, early RFFIT procedures were plagued by problems of non-specific viral neutralization at the lowest dilution levels. Although these problems have been largely resolved by better quality control and more modern laboratory procedures, they have had a lasting influence on the formulation of rabies vaccination guidelines. In particular, this has resulted in the establishment of certain safety factors in testing, such as using a 100% endpoint determination (0/20 infected fields) rather than a 50% endpoint determination (10/20 infected fields) in evaluating samples at the 1:5 dilution.

In order to get around the original problem of non-specific viral neutralization at low serum dilutions, WHO focused its initial attention on dilutions at which this problem was no longer an issue. This turned out to be at dilutions

greater than approximately 1:25, the very next dilution in the fivefold series. WHO then doubled this value to provide an added safety margin. As a result, WHO recommendations for evidence of an adequate response to rabies vaccination were based on antibody concentrations associated with a 1:50 dilution. When set up according to parameters originally established by CDC and using a suitable challenge dose of virus, this corresponds to a rabies-neutralizing antibody concentration of approximately 0.5 IU/mL. The actual rabies titer corresponding to this particular antibody concentration will depend upon the particular parameters used by a given laboratory in setting up the test system. In practice, the WHO recommendation provides a small safety margin over the 1:5 titer recommended by ACIP in the United States.

Only in the United States has the standard for an acceptable response to vaccination been maintained at a titer value corresponding to complete viral neutralization at the 1:5 dilution. Outside of the United States, the WHO recommendation of a minimum rabies-neutralizing antibody concentration of 0.5 IU/mL is essentially recognized as a benchmark for establishing that a satisfactory immune response has taken place following rabies vaccination. Even in neighboring Canada, recommendations for an adequate response to rabies vaccination made by the National Advisory Committee on Immunization are based on the WHO standard.

Persons trying to understand recommendations regarding RFFIT determinations made outside of the United States should keep these points in mind (note: minor differences in ACIP and WHO recommendations also extend to other aspects of rabies prophylaxis). In addition to a titer value, some laboratories in this country may also provide information on antibody concentration, if requested. In fact, the current ACIP recommendations actually state that small differences in the reported values of rabies-neutralizing antibodies are *most properly reported according to a standard as IU/mL*. Indeed, proposals for a generalized conversion to the WHO standards are being strongly considered by public health authorities in this country.

Rabies Titer Determinations

Although the RFFIT determination of a rabies-neutralizing antibody titer has been presented in this article as a relatively straightforward and simple analytical procedure, it is actually an extremely complex test, requiring considerable technical expertise, meticulous preparations, and assiduous quality control for ensuring accurate results. Very few laboratories are certified to perform RFFIT determinations. While a limited number of facilities may provide rabies serology testing for a discrete population (e.g., employees, state residents, military personnel), only two laboratories in the country are certified to perform RFFIT determinations for the public at large: Kansas State University and Atlanta Health Associates. Persons interested in obtaining a rabies-neutralizing antibody titer (either a qualitative screening test or a quantitative endpoint determination) should contact their primary healthcare provider, local or state public health officials, or one of these two laboratories for additional details.

A primary healthcare provider, who may not be knowledgeable about subtle nuances in rabies serology testing procedures,

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is likely to rely on a commercial laboratory to determine where samples should be sent and what tests should be ordered. Although such laboratories perform many routine in-house blood tests, they generally contract with participating facilities for tests that must be sent out. In this regard, it is very important that both physicians and laboratory personnel be advised that you are seeking a RFFIT determination and that one of the two testing facilities listed below be specified. If not, samples may very well be sent out for an enzyme-linked immunosorbent assay (ELISA) determination of antibody levels. The ELISA is not a neutralization test and does not measure the same neutralizing antibodies that the RFFIT does. ELISA results are *not* equivalent to the rabies titer determined by a RFFIT and are *not* reliable for making decisions pertaining to one's vaccination status.

Finally, it should be noted that some commercial laboratories might not be amenable to sending blood tests out to laboratories that are not in their participating network. As such, it is a good idea to call the laboratory ahead of time in order to verify that they will, in fact, send a serum sample to one of the appropriate testing facilities listed below. If they will not send a sample out to a non-affiliated laboratory, a suitable alternative is to have your physician write an order for rabies serology testing that can be submitted to the clinical pathology laboratory of a local hospital, which will almost assuredly comply. After obtaining a suitable blood sample, the laboratory will overnight the required aliquot of serum to the appropriate facility, provided you bring the requisite forms and contact information. The necessary RFFIT serology forms are available online at the websites of the individual facilities listed below.

Veterinary Diagnostic Laboratory
Mosier Hall O-245
1800 Denison Avenue
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas 66506-5601
Phone: 785-532-4483/ 866-512-5650
Fax: 785-532-4474
<http://www.vet.k-state.edu/depts/dmp/service/rabies/guidline.htm>

Atlanta Health Associates
309 Pirkle Ferry Road, Suite D-300
Cumming, Georgia 30040
Phone: 770-205-9091/800-717-5612
Fax: 770-205-9021
<http://www.atlantahealth.net/>

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