SYSTEMATIC REVIEW OF NEUTRALIZING ANTIBODY RESPONSE FOLLOWING RABIES PRE-EXPOSURE VACCINATION AND ADHERENCE TO NATIONAL RECOMMENDATIONS AMONG PERSONS WITH INCREASED RISK OF RABIES EXPOSURE

By

JESSE D. BLANTON

(Under the Direction of JOEL LEE)

ABSTRACT

Animal health workers are a high-risk population for rabies exposure. Rabies prevention recommendations have been developed for this population; however, adherence to these recommendations is not thought to be high based on previous studies. Over the course of two manuscripts this dissertation will investigate the level of rabies exposure risk among animal health workers and their adherence to current recommendations regarding rabies vaccination and serological monitoring. In addition, a systematic review will provide evidence regarding the current pre-exposure vaccination (preEV) administration schedules and alternative routes and schedules evaluated over the previous 50 years.

Over 2,300 persons participated in the survey of animal health providers consisting of animal control officers, veterinarians, veterinary technicians, and wildlife rehabilitators. Bite rates were very high in this population (0.77 bites / person year), as well as potential rabies exposures resulting in PEP (1.07/100 person years). Veterinarians reported a high

rate of rabies vaccination (98%); however, 20-30% of the other groups had never been vaccinated. Similarly 30-40% of all groups were not up-to-date on serological monitoring. Awareness of an employer policy requiring rabies vaccination or serological monitoring was strongly associated with adherence to recommendations.

A total of 51 articles were selected for the systematic review after critical assessment. Primary seroconversion rates (SCR) for cohorts receiving vaccine by the intramuscular route were high and consistent regardless of the vaccine or regimen received. In contrast, cohorts receiving vaccine by the intradermal route reported lower SCRs, and significant heterogeneity was identified between cohorts. However, among cohorts receiving a booster vaccine dose, all subjects responded with an anamnestic response regardless of vaccine, route, regimen, time since vaccination, or titer at time of booster.

The findings of these studies will help develop future recommendations related to rabies preEV. In particular, targeted outreach to high-risk groups including their training institutions and employers may improve adherence to rabies vaccination recommendations. Presentation of these recommendations to national advisory committees may also be of value to update current vaccination recommendations from the 21-28 day schedule to a shortened 7-day schedule, and re-evaluate the frequency of routine serological monitoring among certain risk populations.

INDEX WORDS: Rabies, vaccination, animal health worker, systematic review

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By

JESSE D. BLANTON

B.S. UNIVERSITY OF GEORGIA, ATHENS, 2001

MPH, EMORY UNIVERSITY, ATLANTA, 2003

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirement for the Degree

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By

JESSE D. BLANTON

Major Professor: Joel Lee

Committee: Mark Ebell
Zhen Fu
Stephanie Shwiff

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2017

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CHAPTER 1: INTRODUCTION

OVERVIEW

Human rabies cases have become rare in the United States; however, exposures to potentially rabid wildlife continues to be pervasive[1]. This presents a particular hazard to animal health providers who routinely have contact with animals[2]. While basic infection control practices are the first line of defense in preventing exposures to rabies[3], secondary prevention consists of rabies pre-exposure vaccination (preEV) and postexposure prophyalxis (PEP)[4]. However, adherence to these Advisory Committee on Immunization Practices (ACIP) recommendations is not consistent across different sectors of animal health providers. Several reports have found that rabies preEV rates are low among some high-risk populations and anecdotal reports have suggested that this is an ongoing issue[5]. Adherence to ACIP rabies recommendations, such as routine serological monitoring every six months to two years depending on a person's risk group, is expected to be lower. In parallel, ACIP recommendations related to rabies preEV have not been significantly updated over the past 30-40 years, since modern cell culture vaccines were introduced[6]. Recent evaluations of alternative preEV schedules and duration of immunity may simplify vaccination and help increase adherence to vaccination and serological monitoring recommendations.

Unlike other occupational vaccines that are recommended to protect both the person vaccinated and prevent infection to others in the community, rabies preEV is primarily focused on preventing infection in the person exposed[7]. While exposures to

rabies should be recognized and result in PEP, infection with rabies is almost always fatal if medical intervention is not sought. For persons not previously vaccinated against rabies, PEP requires 4-doses of vaccine and human rabies immunoglobulin. A standard PEP regimen can cost in excess of $3,500 for biologics alone resulting in significant financial impacts on individuals if they are not insured, or employers who may be responsible for ensuring vaccination is provided to employees after an occupational exposure[8]. The cost to previously vaccinated persons, who would require only two booster doses of rabies vaccine, but no human rabies immunoglobulin, is valued at approximately $500. In addition, to directly protecting individual persons at risk of rabies exposure, changes in recommendations are likely to have significant financial impacts as well.

This dissertation presents two manuscripts that examines the current risk among animal health workers and their adherence to current preEV recommendations and the status of research on primary rabies vaccination schedules. A survey of persons who are recommended for preEV due to their increased risk of rabies exposure was conducted to determine rates of potential rabies exposures and to evaluate their adherence to current ACIP recommendations. This survey evaluated several factors that may play a role in exposure rates and individual practices related to vaccination and serological monitoring. Secondly, to determine the current status of research on rabies preEV administration schedules and the duration of immunity, a systematic review of the literature was conducted. In particular this review compared seroconversion rates (SCR) and geometric mean titers (GMT) generated between study cohorts receiving different preEV regimens.

In the United States, the current ACIP recommendations on human rabies prevention were last updated in 2008 and will be under review in the near future. This research project presents information on rabies preEV that will be helpful in reviewing the effectiveness of current recommendations and provide evidence to update future recommendations related to the quantification of risk in different populations, preEV administration schedules, and serological monitoring guidelines. Changes in ACIP recommendations would directly affect more than 200,000 animal health workers[9]. Furthermore, changes to the preEV schedule will have an impact on international travelers, academic researchers, and other groups that represent lower risk groups that may be recommended to receive preEV.

OBJECTIVES

The primary objectives of this research project is to estimate the potential exposure rate among persons at high risk of rabies exposure, determine their utilization of rabies preEV, identify potential risk factors association with non-adherence to current recommendations, and explore current literature for evidence on the longevity of rabies vaccination and alternate routes that may simplify or improve adherence to vaccination and serological monitoring guidelines. These objectives were divided between two studies as follows:

1. The frequency that animal health workers are potentially exposed to rabies, and how they adhere to current ACIP recommendations to receive preEV and participate in routine serological monitoring, and if adherence was affected by individual demographics, knowledge, or other factors.

2. Review of the existing literature for available evidence on alternative administration schedules and routes for rabies preEV, including how these alternatives compare to current recommendations in regard to SCRs and GMTs over time. Also included is how persons who receive rabies vaccination respond to booster vaccination.

BRIEF OVERVIEW OF METHODS

Two methodologic approaches will be utilized to address the stated objectives. First, a survey of persons at risk of rabies exposure will be conducted. This survey will target at-risk persons, based upon their occupational activities. Enrollment announcements will be distributed through veterinarian, veterinary technician, animal control, and wildlife rehabilitator professional organizations to participate in a self-administered anonymous web-based survey. The survey will collect basic demographic information, knowledge of rabies vaccination recommendations, current practices, and exposure histories. A descriptive analysis of results will be conducted. In addition, a logistic regression model will be conducted with current adherence to vaccination and serologic monitoring as the two outcomes of interest.

Another approach will be used to evaluate preEV response following rabies vaccination, which will involve conducting a systematic review of the literature. A review protocol based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines was developed. Search terms for immune responses to rabies preEV were developed. Information was collected from each study on vaccine

type, route, schedule, SCR, and GMT over study period. Heterogeneity of antibody responses was evaluated across studies before additional meta-analysis is conducted.

OUTLINE

Chapter Two describes a review of the current literature related to rabies, its current epidemiology and risk populations, prevention guidelines, and vaccines. Chapters Three and Four present two manuscripts that will be submitted to peer-reviewed journals. The first manuscript provides the results of the survey of persons at risk of rabies exposure and their vaccination practices. The second manuscript presents the systematic review and meta-analysis of anti-rabies antibody response to rabies vaccination. A fifth chapter presents a discussion of the findings of these manuscripts, their public health significance, and future work that should result from this analysis.

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CHAPTER 2: LITERATURE REVIEW

LYSSAVIRUSES AND RABIES

Lyssaviruses, the causative agents of the disease rabies, are negative-sense single-stranded RNA viruses in the Family Rahbdoviridae[1]. Currently 17 recognized or putative *Lyssaviruses* are distributed globally[2,3]. However, *rabies virus* is the only species in the genus that is pandemic in distribution, is the only recognized species currently in circulation in the New World, and accounts for most of the global rabies burden in humans and animals[4]. *Lyssaviruses* can infect all mammals resulting in progressive encephalitis that nearly always results in death. The virus is neurotropic, moving from the exposure site to the brain exclusively via peripheral nerves[1]. Once the virus reaches the brain it begins to replicate before migrating to the salivary glands. Virus shedding in saliva is intermittent, but may begin several days before the onset of clinical signs[5,6].

Exposure to rabies occurs through direct contact with infectious saliva or nervous tissue, either from a bite or contamination of open wounds or mucosal tissue with these materials[7]. Following an exposure, the incubation period is variable depending on the proximity of the exposure site to the central nervous system. Silent or sub-clinical infections, as indicated by detection of rabies virus neutralizing antibodies (RVNA) without the onset of clinical illness, are documented in humans and animals and may represent the outcome of the majority of rabies exposures[8-11]. Prior to the development of rabies postexposure prophylaxis (PEP), the probability of mortality was estimated

between 5-70% depending on the site of exposure (e.g. bite to hand versus head)[12]. However, once clinical signs have begun, an outcome of death is near universal.

On average the incubation period in humans is one to three months, however incubation periods ranging from a few days to several years have been reported [1,13]. Onset begins with influenza-like symptoms, often with paresthesia associated with the exposure site. These early signs progress into more acute neurologic symptoms including: anxiety, confusion, agitation, abnormal behaviors, and/or hallucinations. This acute phase may last for 2-10 days[1]. Classical signs of rabies such as hypersalivation, hydrophobia, and aerophobia are common at this stage. Following the acute phase there is a rapid deterioration in neurologic status resulting in coma followed by death[1]. Once signs of rabies have begun there is no recognized treatment and care for patients is primarily palliative[14]. While some success has been observed with experimental treatment protocols, standardization and validation of these protocols is ongoing[15]. The most cost-effective way of preventing rabies will likely remain pre-exposure vaccination (preEV) or PEP for the foreseeable future[15].

RABIES EPIDEMIOLOGY

Despite effective means for its control, rabies remains a classical zoonotic disease with a worldwide distribution. Globally an estimated 60,000 persons die of rabies infection; with the majority of human rabies cases occurring in Africa and Asia[4]. In addition, rabies disproportionately affects children and poor populations[16]. Another 30 million persons are estimated to receive rabies PEP annually due to potential rabies exposures. The global cost of rabies is estimated to exceed $5 billion USD annually from premature deaths, cost of biologics, and lost income while receiving PEP[4]. Dogs are

responsible for nearly all reported human rabies cases globally, however, terrestrial wildlife reservoirs have been identified on every continent with the exception of Australia and Antarctica (with Antarctica being the only continent free of Lyssaviruses)[17]. Where canine rabies has been eliminated, these wildlife reservoirs continue to play an important role in maintaining the virus so that rabies exposure risks continue, particularly for populations at increased risk of contact with animals (e.g. veterinarians, wildlife biologists, etc).

While canine rabies was eliminated in the United States in 2005, more than 6,500 cases of animal rabies are still reported in the US annually (93% in wildlife). In addition 2-3 human cases are reported each year [18,19]. Eight terrestrial rabies virus variants are maintained by four species: North Central Skunk Rabies Virus Variant (RVV), South Central Skunk RVV, California Skunk RVV, Arctic Fox RVV, Arizona Gray Fox RVV, Texas Gray Fox RVV, Raccoon RVV, and Mongoose RVV[20]. Collectively these RVV broadly cover most of the United States and Puerto Rico. However, because of the broad distribution and movement of bats, no state is considered free of the RVVs associated with more than 23 bat species in the US. Despite the high mutation rates of RNA viruses, RVV and their association with reservoir species have remained relatively stable over time[21], but host shift events and establishment of new RVV in new reservoirs has been reported[22]. At least within bat RVV, relatedness between species has been closely associated with cross-species emergence of new variants[23].

Since 1990, the majority of reported human cases in the US have been associated with bats[20]. Bats are believed to be associated with more human deaths from rabies because their bites are less traumatic and therefore persons are less likely to seek medical care and treatment [24]. While bat RVVs are responsible for most human rabies

cases, terrestrial mammals are believed to account for most potential exposures resulting in PEP[25]. In particular, despite the lower risk from cats and dogs since the canine RVV has been eliminated, between 30-50% of PEP is believe to still be administered after exposure to these species[25,26].

HIGH-RISK EXPOSURE GROUPS

While the national incidence of rabies exposures resulting in PEP is estimated to be 8.5 / 100,000 persons, the risk of exposure among persons who have greater contact with animals (either due to occupational or leisure activities) are much higher [25]. Based on a person's exposure risk the Advisory Committee on Immunization Practices (ACIP) has developed four risk categories that help refine specific recommendations for these groups: continuous (e.g. rabies laboratory workers), frequent (e.g. animal health providers working in terrestrial rabies endemic areas), infrequent (e.g. animal health providers in low endemic areas, some international travelers), and rare (e.g. the general population at large)[7].

In general, zoonotic diseases pose an inherent risk to animal health workers and other occupations with frequent animal contact. Zoonotic infections that may be encountered in an occupational setting range from mild infections like dermatophytosis (ringworm), moderate infections like *Campylobacter*, to severe infections like rabies [27]. While the risk of disease transmission from humans to animals is possible, anthropozoonosis, or animal to human transmission, presents the greatest occupational risk to workers. Most studies of occupational exposure to zoonotic diseases have examined veterinary health workers. Surveys of veterinarians have reported that an estimated 35-64% contract at least one zoonotic infection during their career [28,29].

Another study among animal control officers (ACOs) in New Mexico found an overall bite rate of 2.5/working year, which was estimated to be 175-500 times higher than the national bite rate [30]. Exposures to suspected rabid animals among veterinarians have been reported in as many as 20.8% of veterinarians surveyed in Oregon, and is likely higher in states with a greater wildlife rabies burden [29]. Just as human healthcare providers should implement routine vaccination and prevention measures, animal healthcare providers have an obligation, and often a legal liability, to ensure they and their staff take appropriate precautions to prevent zoonotic disease transmission [27]. In some cases zoonotic illnesses may continue to present a risk of transmission in the human healthcare setting when sick individuals present to their human healthcare providers for consultation [29,31,32]. Standard precautions recommended by National Association for State Public Health Veterinarians (NASPHV) for veterinary personnel include recommendations for several vaccinations to prevent direct infection (i.e. rabies and tetanus) or infection to animals that could cause public health threats (i.e. influenza) [33].

Despite ACIP and NASPHV recommendations, varied degrees of adherence among animal health providers are reported. Most veterinary colleges require students to receive rabies preEV before beginning clinical coursework. Subsequently rabies vaccination coverage among veterinarians is relatively high (>85%) [29,34,35]. However, similar surveys have reported much lower coverage amongst other occupational groups. One survey reported that only 42% of wildlife rehabilitators in North Carolina had received rabies vaccination, while another survey reported only 37% of animal control officers in New Mexico had been vaccinated [30,36]. The rate of vaccination also varies among staff categories working within veterinary clinics. In one study, as few as 17.5%

of other non-veterinarian staff (e.g. kennel workers, volunteers, etc.) working in surveyed clinics had been vaccinated [34]. A survey of veterinary clinics in West Virginia found 45% of clinics had a policy requiring veterinarians to be vaccinated against rabies, but only 15% required the same of technicians [35]. Relatively few studies have evaluated adherence to serological monitoring recommendations, but have generally found even lower adherence[35]. Potential factors that have been associated with adherence to rabies vaccination recommendations and serological monitoring are the high costs of rabies vaccines and length of employment in a position [34]. The effect of individual cost and subsidy, through insurance or employment benefits, on adherence to vaccination and serological monitoring recommendations has not been explored.

RABIES PREVENTION

Despite the near universal death rate associated with rabies, it is essentially 100% preventable with the prompt and accurate administration of rabies PEP. The primary objective of rabies prophylaxis is to neutralize rabies virus before it invades immune privileged neuronal cells[37]. For persons without rabies preEV, prophylaxis consists of thorough cleaning of the bite or exposure site, administration of rabies immunoglobulin (RIG) at 20 IU/kg around the wound, and four doses of rabies vaccine (administered on days 0, 3, 7, and 14). A fifth dose of vaccine, on day 28, is recommended if a patient is immunocompromised [7,38]. Alternative intramuscular (IM) and intradermal (ID) schedules have also been recommended by WHO[39]. If a person has been previously vaccinated against rabies only two doses of vaccine are administered on days 0 and 3, and RIG is contraindicated.

Rabies preEV is only recommended for continuous, frequent, and infrequent risk categories [7]. PreEV consists of three doses of rabies vaccine administered on days 0, 7, and 21 or 28. Additional serologic monitoring is recommended for persons in the continuous and frequent risk categories (i.e. every 6 months or 2 years, respectively) to ensure a detectable RVNA level is maintained [7]. PreEV does not preclude the need for PEP, but simplifies the intervention by eliminating the need for RIG and reducing the number of vaccine doses administered from four to two (administered on days 0 and 3). In addition to reducing the need for additional biologics, PEP of previously vaccinated persons may reduce biologics costs by more than 400%[40].

Confirmation of sero-conversion is not routinely recommended after rabies vaccination. While higher titers have been roughly correlated with an increased probability of protection against rabies infection, no minimum protective anti-rabies antibody titer has been identified [7]. In the United States, the current ACIP guidelines for rabies serological monitoring in persons in the continuous or frequent risk categories recommend a vaccine booster when a person's titer falls below complete neutralization at a serum dilution of 1:5 (approximately 0.1-0.2 IU/mL) using the Rapid Fluorescent Focus Inhibition Test (RFFIT) [7]. Alternatively the World Health Organization (WHO) uses a more conservative guideline, recommending a booster when a person's titer drops below 0.5 IU/mL (complete neutralization at a serum dilution of 1:25) [39]. Regardless of the titer values used for recommending a booster, serologic monitoring of RVNA remains a surrogate measurement to determine the overall anti-rabies immune status [7,41]. While these recommendations have changed over the years, rabies vaccine boosters and serological monitoring remain important components. Prior to the availability of highly potent cell culture rabies vaccines a booster vaccine was

recommended every 2-3 years for persons at high risk of rabies exposure rather than serological monitoring [42]. When the human diploid cell vaccine (HDCV), which is safer and more potent than previously available vaccines was licensed in 1980, recommendations were updated to recognize serological testing as an alternative to routine vaccine boosters [43]. ACIP recommendations were updated in 1984 in favor of serological monitoring. However, these recommendations have been complicated by the increased need for frequent blood sampling and limited laboratory capacity to conduct RFFIT testing making adherence to recommendations difficult [44].

RABIES VACCINES

Considerable evolution has occurred in the development of rabies vaccines since Louis Pasteur's first application of rabies PEP in 1885. The earliest rabies vaccines were largely primary nervous tissue vaccines consisting of brain homogenate from animals infected with various rabies virus vaccine strains typically chemically inactivated[37]. These vaccines were associated with various severe adverse events, including cases of vaccine induced rabies from poorly inactivated lots [45]. Despite recommendations against the use of these vaccines by WHO, they still persist in several countries largely due to the increased cost of modern vaccines[39]. Availability of modern tissue culture vaccines for rabies did not begin until the 1970s and the first tissue culture based vaccine became widely available on the US market in 1980. WHO recommends cell culture vaccines with a potency of ≥2.5IU be used for rabies preEV or PEP. In general, there are four categories of rabies vaccine available in the global market that meet those recommendations: Human Diploid Cell Vaccine (HDCV), Purified Chick Embryo Cell Vaccine (PCECV), Purified Vero Cell Rabies Vaccine (PVRV), and Purified Duck Embryo

Vaccine (PDEV)[37]. Only HDCV and PCECV are currently licensed and available in the United States[7]. These vaccines have been documented to produce high SCRs and produce very low rates of adverse reactions[46,47]. Local reactions are the most commonly reported adverse event to rabies vaccines. However, rare neurological (e.g. seizures) adverse events have been reported [7].

While several variations on the number of doses and administration schedule have been proposed for PEP, recommendations for preEV have been fairly consistent since the early 1980s (3 doses of vaccine administered on day 0, 7, and 21 or 28). The ACIP restricts administration to the IM route, but WHO recommends both IM and ID administration routes as acceptable[7,39]. Optimally, preEV would consist of a single dose of vaccine that would consistently produce a high RVNA response among all persons vaccinated with a long duration of immunity. Several studies have compared administration routes, schedules, and doses for preEV. These early studies found at least three doses of HDCV administered over a month were needed to elicit 100% seroconversion among vaccines[48]. Many studies have found preEV administered through the ID route to produce lower antibody responses compared to IM administration, though the difference is not likely to be biologically relevant[37,49]. The ID administration route for rabies preEV has been documented to produce a significantly stronger cell mediated response compared to the IM route, when a comparable dosage is administered[50,51]. However, the recommended ID schedule is one-tenth of the dose of the IM regimen, which may account for the lower RVNA response seen in most studies[39].

In addition to the primary response to vaccination, several studies have looked at the long-term persistence of rabies neutralizing antibodies. Most studies have been

restricted to relatively short follow-up periods ranging from 1-3 years or have involved schedules that included one or more boosters [52-54]. In general, two years after completing the 3-dose pre-exposure vaccination series >93% of persons will maintain an adequate antibody titer (>0.11 IU/mL) as recommended by ACIP [55], lower seroconversion rates are expected if evaluating based on the more conservative WHO recommendations (>0.5 IU/mL). Several studies have suggested long-term persistence of rabies antibodies (>8 years), but have generally all involved administration of a routine booster at 1-year [44,48].

FUTURE OF RABIES VACCINATION

Currently available vaccines are highly efficacious with low rates of adverse events. Future development of rabies vaccines and administration recommendations are likely to focus on containing associated costs of these vaccines while expanding coverage to at-risk populations. ID vaccination routes present an existing opportunity to reduce preEV costs and are slowly gaining acceptance globally[56]. However, off-label usage of single-dose vials for multi-dose ID administration impedes adoption in many countries. Packaging for ID administration is similar to those currently licensed for ID influenza vaccine and might increase the likelihood for adoption and address concerns about proper ID administration[57]. In addition, reduced dose and/or shorter immunization schedules for primary vaccination may simplify vaccination and increase adherence among persons recommended to receive preEV.

Extension of rabies preEV to additional at-risk population may have the potential to decrease the burden of rabies. In particular, preEV of children in rabies endemic areas where access to PEP is limited may be an effective way to reduce unnecessary

rabies deaths. However, the current cost of vaccine to support programs that would administer to these populations has been cost-prohibitive in pilot studies conducted to date[58]. Additional research to evaluate the effectiveness of these programs will likely continue.

Finally, development of several recombinant systems for rabies virus have opened up pathways to develop novel rabies vaccines that may simplify administration, reduce costs, and improve immunologic response[59]. Some of these vaccines are in development, but practical utilization for human vaccination is further on the horizon. Current vaccines do not protect against all lyssaviruses and development of a pan-lyssavirus vaccine may be important, particularly in areas of Africa and Asia where these lyssaviruses are in circulation among bat populations [60,61]. Similarly bi- or multi-valent vaccines that offer protection against rabies and other co-endemic diseases may improve vaccination coverage and be an effective way of incorporating rabies vaccination into other vaccination programs[62,63].

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CHAPTER 3: RABIES EXPOSURES AND PRE-EXPOSURE VACCINATION PRACTICES AMONG PERSONS WITH INCREASED RISK OF RABIES EXPOSURE [*]

[*] Blanton, Jesse D. To be submitted to the Journal of the American Veterinary Medical Association

ABSTRACT

Objective – Identify knowledge and practices related to rabies vaccination and serological monitoring practices among animal care workers.

Design – Cross-sectional survey

Sample – 2,334 animal care workers (i.e. animal control workers, veterinarians, veterinary technicians, and wildlife rehabilitators)

Procedures – Participants were contacted through relevant professional organizations to participate in an anonymous web-based survey. The survey collected data on demographic and occupational information, animal handling and potential rabies exposure information, and individual rabies vaccination and serologic monitoring practices. Comparisons of animal bite and rabies exposure risks were made between occupational groups based on exposure rates. Multiple logistic regression was used to evaluate factors associated with rabies vaccination status and adherence to rabies serologic monitoring.

Results – Overall, respondents reported 0.77 animal bites per person year or 0.06 bites per 1,000 animals handled. Potential rabies exposures resulting in postexposure prophylaxis were reported at 1.07 / 100 person years with the highest rate reported among wildlife rehabilitators. Over 98% of veterinarians reported being previously vaccinated against rabies. However, 20-30% of the other occupational groups had never received vaccine against rabies. Conversely, 30-40% of all groups were not current on their serological monitoring as recommended by the Advisory Committee on Immunization Practices (ACIP). Animal control officers and wildlife rehabilitators that

were aware of an employer policy requiring rabies vaccination were 26 times more likely to be vaccinated against rabies compared to veterinary technicians (who reported the lowest rabies vaccination rate). Similarly, all respondents were 5.5 times more likely to be current on serological monitoring if they were aware of an employer requirement compared to those working for employers without such a policy.

Conclusions –With the exception of the high rabies vaccination rate among veterinarians, improvements in rabies vaccination and serological monitoring are needed among animal care workers to improve adherence to ACIP recommendations. Several factors were associated with a history of rabies vaccination and current serologic monitoring, however, employer polices requiring these practices had the largest effect towards improving adherence. Improving adherence to these recommendations is important given the high reported rates of animal bites and potential rabies exposure.

INTRODUCTION

Persons with occupational contact with animals are inherently at greater risk for exposures to zoonotic diseases than the general population. Between 35-64% of veterinarians have reported acquiring a zoonotic infection over the course of their career.[1,2] In addition, animal bites are one of the most common injuries received by animal care workers. Greater than 60% of veterinarians have reported a history of an animal bite over the course of their career in studies representing subjects from the Americas, Australia, and Europe.[3-7] Several studies have examined the rate of bite injuries among other occupational groups that work with animals. A 1984 study reported 40% of animal control workers in New Mexico had a history of an animal bite (~1.8 bites per 1,000 animals handled), or a bite rate 175-500 times higher than the

general population.[8] Bites were reported among 98% of veterinary nurses and technicians at an international conference in Australia, and bites resulting in an infection were reported among 44% of technicians in a survey of veterinary facilities in Minnesota.[9,10] In comparison, a national survey conducted between 2001-2003 found approximately 1.5% of the US population are victims of dog bites annually, with 19% seeking some kind of medical care[11]. No studies have estimated the rate of bites from multiple species in the US; however, emergency room visits from all animal bites have been reported between 125-135/100,000 visits[12,13].

Fewer studies have examined risk factors associated with the increased risk of animal-related injuries. However, increased age and more experience working with animals has generally been identified as negatively associated with bite rates among all occupational groups [3,8,14]. While these bites may result in severe physical trauma and localized infections requiring medical attention, they also constitute potential rabies exposures in endemic areas with nearly 21% of veterinarians reporting contact with a suspect rabid animal in one study [15,16].

The Advisory Committee for Immunization Practices (ACIP) categorizes rabies risk as continuous, frequent, infrequent, or rare.[17] Animal care workers are categorized in the frequent or infrequent category depending on whether rabies is endemic in terrestrial animals where they work (i.e. frequent where terrestrial rabies is present, infrequent where it is not). Both risk categories are recommended to receive primary rabies vaccination, however, persons with frequent exposure risk are also recommended to undergo routine serological monitoring for anti-rabies antibodies.[17] Primary rabies vaccination consists of three doses of rabies vaccine administered on days 0, 7, and 21 or 28. While prior vaccination does not preclude the need for additional care in the event

of a rabies exposure, it simplifies the postexposure prophylaxis (PEP) regimen (only two booster vaccine doses on days 0 and 3 and no human rabies immune globulin (RIG) compared to RIG plus four or five doses of vaccine).[17] Serological monitoring every 2-years is recommended for those in the frequent risk category to ensure the presence of a primed immune response. While no rabies neutralizing antibody (RNA) level has been identified as protective in persons who have been previously vaccinated against rabies, complete neutralization at a serum dilution of 1:5 by the rapid fluorescent focus inhibition test (RFFIT), equivalent to 0.1-0.2 IU/mL, has been identified by ACIP as minimum evidence of an immunologic response.[17] If RNA drops below this level when serological monitoring is conducted, a single dose of rabies vaccine is administered to boost the immune response.

Despite these inherent risks and recommendations, adherence to ACIP recommendations is variable among animal care workers. While veterinarians have reported relatively high rabies vaccination rates (>80%), lower rates are generally reported among other occupational groups with frequent and infrequent rabies exposure risks.[10,16] Lower rates of vaccination (typically around 30-50%) have been reported among veterinary technicians.[10,18] Generally less than a third of any veterinary facility staff have reported receiving a rabies titer within the past two years.[10,18] Veterinary facility policies may not encourage adherence to these recommendations either. A survey of veterinary facilities in West Virginia found only 45% of all facilities (52% in raccoon rabies endemic counties / 38% in terrestrial rabies free counties) had a policy requiring veterinarians receive primary rabies vaccination, compared to only 15% (24% / 7%) having the identical policy for veterinary technicians.[18] Similarly only 25% of

facilities in raccoon rabies endemic counties had a policy regarding serologic monitoring veterinarians, compared to 15% for veterinary technicians.

The purpose of this study was to assess the knowledge, attitudes, and practices of persons in various occupational activities that place them at increased risk for bite injuries and potential rabies exposure on a national scale. In particular this includes veterinarians, veterinary technicians, animal control officers, and wildlife rehabilitators.

METHODS

STUDY DESIGN

The study was designed as a descriptive cross-sectional survey of persons with occupational activities that increase their risk of rabies exposure. Specifically, veterinarians, veterinary technicians, animal control officers, and wildlife rehabilitators were contacted through their respective professional organizations. The study protocol was reviewed and approved by the Institutional Review Board of the University of Georgia (IRB# 3431)

The survey was divided into five sections. A demographic section collected information on respondent's age, sex, state of residence, level of education, and household income. Another section collected information on the respondent's knowledge of rabies infection and vaccination recommendations was included. In the third section, information was collected on the respondent's type and length of employment, frequency of working with animals, and information on potential rabies exposures. A fourth section collected information on the respondent's rabies vaccination history and serological monitoring practices. And finally, a section where a respondent's

different willingness to pay and risk attitude scenarios were presented. This paper focuses only on findings from the first four sections.

Four professional organizations (American Veterinary Medical Association (AVMA), National Animal Control and Care Association (NACA), National Association of Veterinary Technicians of America (NAVTA), and National Wildlife Rehabilitators Association (NWRA)) were contacted to assist with recruitment from their membership. The survey was provided for review to executive members of each organization and suggestions incorporated into the survey before the final web-based version was developed in Survey Monkey [19].

A recruitment letter and web-link to the survey was provided to each professional organization where it was distributed to members by email distribution list or in electronic newsletter. The recruitment letters were distributed towards the end of May 2016 and the survey remained open until July 31, 2016. One reminder announcement was distributed by all organizations approximately 5-6 weeks after the initial recruitment notice.

ANALYSIS

All data were exported from Survey Monkey and entered into a Microsoft Access database for cleaning and analysis using statistical analysis software[20]. Variables were compared between each occupational group (animal control, veterinarians, veterinary technicians, and wildlife rehabilitators), between persons with and without rabies vaccination, and between persons who did or did not adhere to ACIP rabies serological monitoring guidelines[17]. Respondent's state of residence was used to determine the rabies reservoir region they lived in according to national surveillance designations[21].

Respondents were questioned about the number of animals they have contact with each week and the number of animals with suspect neurological disorders they have contact with each month to limit recall bias. These rates were annualized for additional calculations. A hypothetical gamble scenario was used to assess individual risk attitudes. Nested questions about an individual's willingness to accept a new employment opportunity were presented. Subsequent questions adjusted the probabilities of increasing or decreasing the person's current pay and the total relative change in pay. A risk aversion category from one (most risk averse) to four (least risk averse) was assigned based on individual responses[22].

Continuous variables were evaluated for normality based on heteroskedasticity and visual inspection of histograms. Variables were log transformed if substantial deviations from normality were observed. Frequencies were calculated for categorical variables and means and 95% confidence intervals for continuous variables. Geometric means were used for continuous variables that were log transformed. A χ^2 test or t-test was used as appropriate for tests to determine statistical differences between groups. Univariate odds ratios were calculated for the vaccination and serological monitoring status comparison groups. Any variables from this univariate analysis with a p-value $\leq$ 0.1 were entered into a multiple logistic regression model along with potential 2-way interactions between occupational groups and all other variables. Final models were selected using a stepwise process using the likelihood-ratio statistic and individual evaluations for confounding. Multicollinearity was evaluated visually using a correlation matrix between variables and by a weighted regression model to generate eigenvalues and conditional indices. Variables with conditional indices greater than 10, were

considered to represent potential collinearity. For all final statistical tests, p-values <0.05 were considered significant.

RESULTS

A total of 2,919 responses were received from the web-based survey. Based on the estimated distribution provided by the participating professional organizations this represented an estimated response rate of approximately 23% (2,919 / ~12,700). Of these participants, 426 were immediately excluded because they did not complete the survey. An additional 159 participants were excluded because they either indicated they had no animal contact in their position or indicated they were in administrative or teaching positions with very limited animal contact rates. Final analysis was conducted on 2,334 participants representing a completed survey response rate of 18% (Figure 1).

In total, 445 participants identified their current positions as an animal control worker (ACW), 375 as a veterinarian (Vet), 1,357 as a veterinary technician (VT), and 157 as a wildlife rehabilitator (WR; Table 1). The mean age among all participants was 41.8 years (95% confidence interval (CI): 41.3 – 42.3). Veterinary technicians were significantly younger than the other occupational groups at 38.7 years (95% CI: 38.1-39.2). Overall the majority of participants were female (86.8%) with VTs and WRs having a higher proportion of women compared to ACWs and Vets. Approximately 40% to 50% of all respondents resided in a state where the raccoon rabies virus variant is the primary reservoir of rabies. With the exception of ACWs, 85% of the respondents in each group reported a bachelor's degree or higher. The average length of time worked by all respondents in their current position was 10.8 years. Vets and WRs reported significantly longer time in their current position compared to ACWs and VTs. History

of rabies vaccination varied across the different occupational groups with Vets reporting the highest rate (99%), followed by ACWs (78%), WRs (78%), and VTs (69%). Among persons who reported a history of vaccination, adherence to serological monitoring according to ACIP recommendations was lower, with 61% of all respondents considered up to date. Vets reported the lowest proportion of respondents with current monitoring status (55%).

With the exception of WRs, more than 85% of the persons in each group indicated they worked with companion animals (e.g. cats and dogs; Table 2). A high proportion of ACWs and WRs indicated they work with wildlife, and more than 60% in both groups reported working with rabies reservoir species (i.e. raccoons, skunks, foxes, or bats). The geometric mean of animals handled annually was 1,908 (95% CI: 1,829.3 – 1,990.0). While ACWs and WRs handled significantly fewer animals, both groups had higher rates of animal bites compared to Vets and VTs. Overall, ACWs reported 0.77 animal bites per person year, with WRs reporting the highest rate (1.66 bites/person year). Adjusted for the number of animals handled, the overall geometric mean for all respondents was 0.07 bites per 1,000 animals handled (95%CI: 0.06 - 0.08). The majority (56%) of participants had a history of handling a suspect or confirmed rabid animal at some point while in their current position. Overall, ACWs and Vets reported the highest rates of handling a suspect rabid animal at 72% and 61% respectively. However, ACWs and WRs had the highest rates of contact with suspect rabid animal per 1,000 animals handled at 1.24 and 0.54 respectively. Exposures to rabies during these contacts occurred in about 20-30% of the most recently occurring encounters. Overall 21% of respondents reported receiving rabies PEP due to an exposure while in their current position. WRs reported the highest rate of receiving PEP due to an occupational

exposure at 47%. The overall rate of PEP due to an occupational exposure was 1.07 / 100 person years. Cats were the most frequently reported animal involved in potential rabies exposures that resulted in PEP (37%), followed by raccoons (16%), bats (12%), dogs (12%), and livestock (8%).

Knowledge of rabies infectivity and national ACIP recommendations differed significantly between occupational groups. Recognition of saliva as a potentially infectious substance was high (>96%) among all groups (Table 3). However, recognition of nervous tissue as a potentially infectious material was low among all groups, but particularly among ACWs and WRs (56% for both). Furthermore, the proportion of respondents who, incorrectly indicated blood was potentially infectious for rabies ranged between 40%-54%. The participants were asked several questions about current ACIP recommendations for human rabies vaccination. Greater than 77% of respondents recognized the appropriate course of rabies pre-exposure vaccination consisting of 3 doses of vaccine. However, only 15%-35% of participants recognized the appropriate PEP management for a previously vaccinated person (i.e. 2 booster doses of vaccine). Overall less than half (41%) of participants recognized that anti-rabies titers are recommended every 2 years for persons who work with animals in rabies endemic areas. Most (49%-75%) indicated they did not know what titer level is recommended for indicating when a rabies vaccine booster is necessary. Where an appropriate cut-off titer was recognized, nearly twice as many participants in each group identified the World Health Organization (WHO) recommended levels over the ACIP recommendations.[17,23] Knowledge of an existing employer policy requiring rabies vaccination or routine titer monitoring was low across all groups. VTs were the least likely to report an employer

requirement to receive rabies vaccination (18% of VTs) while ACWs and WRs had the highest reported rates (39% and 38% respectively).

Among persons with a vaccination history, 1,005 (57%) were categorized as meeting the ACIP criteria as a frequent risk group and would be recommended to undergo serological monitoring every two years. Of these, 613 (61%) were considered current on their monitoring (i.e. rabies neutralizing titer received within past 2 years). Univariate analysis identified several factors significantly associated with both a history of vaccination and current serological monitoring status (Table 4): age, household income, years in current position, occupational group, history of working with rabies reservoir species, number of animals handled annually, history of handling a suspect rabid animal, and employer policies requiring vaccination/serological monitoring. Vets were 32 times more likely to be vaccinated than VTs and persons with an employer policy requiring vaccination were 24 times more likely to be vaccinated than those without. Respondents with an employer policy requiring serological monitoring were 5 times more likely to have a current titer. Age had an inverse effect for vaccination compared to serological monitoring. Continuous increase in age by year was significantly associated with a higher likelihood of being vaccinated, but a lower likelihood of being current on serological monitoring.

Two multivariable logistic regression models were fit for likelihood of rabies vaccination and likelihood of having a serological monitoring status (Table 5a and 5b). No substantial collinearity between variables in either final model was observed. Controlling for other factors, respondents residing in a state where raccoon rabies virus variant is present were three times more likely to be vaccinated than those living in states free of terrestrial rabies (e.g. rabies only present in bats). With the exception of

Vets, increased education was generally associated with a higher likelihood of vaccination compared to those with a high school degree or less. A higher likelihood of vaccination was identified among ACWs and Vets compared to VTs (1.5 and 33 times higher respectively). Respondents reporting an employer policy requiring vaccination were 32 times more likely to be vaccinated compared to those without a policy (Table 5a). A respondent's level of risk aversion had a significant impact on model fit (log rank χ^2 170.28, p-value <0.0001) and strongly confounded (>10%) the effects of other parameters if removed, so this variable was retained in the final model. While not statistically significant, as respondents became less risk averse, they were more likely to be vaccinated against rabies.

One significant interaction term between the rabies reservoir region and occupational group was associated with serological monitoring status (Table 5b). The interaction term primarily modified the association between WRs in raccoon endemic regions compared to other terrestrial rabies endemic regions (e.g. skunk, fox, or mongoose). WRs in raccoon regions were more than 7 times more likely to not be current on their serologic monitoring compared to those in other terrestrial rabies endemic areas. This effect was not significant for the other occupational groups. Significant, but moderate, effects were observed for income and history of handling rabid animals both associated with an increased likelihood of being current on serological monitoring. A respondent's age and the reported annual number of animals handled were negatively associated. Respondents that reported an employer policy requiring serological monitoring were more than six times as likely to be current on their monitoring. Also similar to the vaccination status model, risk aversion category had a significant impact on model fit (log rank χ^2: 79.63, p-value <0.0001).

DISCUSSION

Reports of animal bites among survey respondents were approximately 50 – 165 times greater than the estimated national bite rate for dogs alone. While some of this increased rate is attributable to the inclusion of multiple species, the increased risk was also observed in the risk of rabies exposure. Among all respondents, potential rabies exposures resulting in PEP were reported at 1.07 per 100 person years. The current estimated rate of PEP in the US is 11.7 per 100,000, making the risk among animal care workers more than 90 times greater than the general population[24].

Recognition of these risks is the foundation for ACIP and NASPHV recommendations. However, basic knowledge of rabies transmission, infectivity, and vaccination recommendations were lacking overall in all occupational groups. Nearly all respondents correctly recognized infectious substances, nearly half also incorrectly identified urine and blood as an infectious substance. While this is lower than response rates seen in similar surveys among the general population it is still particularly high among a cohort of persons with increased animal contact that should be familiar with determining potential rabies exposures[25]. In addition, only 20% of respondents knew the correct course of rabies PEP for persons who had been previously vaccinated and familiarity with serological monitoring recommendations were mixed. Awareness of these clinical recommendations are part of the process of making informed medical decisions with an individual's healthcare providers. Previous studies have documented cases of inappropriate PEP administration in the clinical setting and confusion regarding appropriate titer cut-offs exists even in the scientific literature[26,27]. Based on the documented increased risk of exposure among animal care workers, improved

knowledge may be critical to ensure that they are able to take a more proactive role in their healthcare in the event of an exposure or during occupational health monitoring to ensure appropriate care is provided.

Rabies vaccination rates were significantly different between occupational groups. Nearly all veterinarians were vaccinated against rabies, a finding consistent with prior studies [27]. Rabies vaccination rates ranged between 70-80% in other studies, which have also found the lowest rabies vaccination rates among VTs (as they were in this study). While VTs also had a lower bite and rabies exposure rate, it was not significantly different than that observed for Vets, for whom vaccination is typically required to complete their academic training or enter the workforce. However, likelihood of vaccination was also associated with length of time working in a respondent's position. This may be related to a stronger incentive to get vaccinated as a person becomes more familiar with the risks in their position or may represent a cohort effect where the vaccination coverage increases due to PEP over time from occupational exposures. An opposite effect appears in relation to serological monitoring. Adherence to ACIP recommendations decreases with a respondent's age. Rabies titers often do not change significantly over time and this decrease in adherence with age may represent fatigue in maintaining regular monitoring when no change is noticed over time [28].

Rates of serological monitoring were lower across all animal care workers and nearly a quarter of respondents reported titers should only be monitored every 5 years. Very few human rabies cases have been reported among persons with a history of pre-exposure vaccination and those have typically involved unusual exposure routes (e.g. laboratory exposures) or co-administration with drugs that might affect the immune response (e.g. chloroquine)[29-31]. In addition, some studies have suggested the duration

of immunity for rabies pre-exposure vaccination may exceed 10 years[32]. Given issues with adherence to current serological monitoring recommendations and potentially long lasting immunity, additional cost-effectiveness analysis is warranted to determine the optimal period for monitoring or if monitoring is necessary.

While workplace polices requiring rabies vaccination or serological monitoring appear to have a significant effect on adherence to ACIP recommendations, less than 25% of respondents reported such polices at their employment. Vaccination requirements for veterinary students appear to have been a successful strategy for ensuring a high vaccination level among veterinary cohorts. A similar tactic might be considered, to require rabies vaccination, for training programs focused on ACWs, VTs, and WRs. However, outreach to academic and training programs and employers is warranted to encourage policies reinforcing ACIP recommendations. More work is needed to understand why rabies vaccination policies are not implemented more consistently. In the interim, additional guidance for workplace policies might be considered for ACIP and NASPHV recommendations [33,34].

The results of this survey represent the first attempt to collect information on animal bites, rabies exposure, and adherence to ACIP rabies vaccination recommendations across multiple occupational risk groups and on a national scale. In particular this study reports information obtained from ACWs and WRs, two groups that have rarely been surveyed in previous studies examining rabies exposure risks and vaccination practices. Identifying factors that may be associated with adherence to current ACIP recommendations may be important in developing interventions that reduce risk in a cost-effective manner. However, several limitations should be noted. As with any cross sectional survey, recall bias is a concern when seeking information on

prior exposures. Shorter recall periods for information with high numbers of events (e.g. number of animals handled weekly) were used in an attempt to minimize this. Generally more heaping (i.e. responses ending in units of 0 or 5) was observed for questions that involved high frequency events compared to less frequent events such as bites. This suggests respondents provided more generalized responses for high frequency events. In addition, the recruitment process through professional organizations likely resulted in some selection bias. However, participation in a professional organization is likely to select for persons that are more engaged in their profession and may receive more information, education, and communication services compared to those not involved in a professional organization. The method of recruitment made exact determination of a response rate difficult, but was likely around 18% based on estimates of distribution list members from each organization. While this response rate was low it was comparable to some previous studies presented here and appeared to be representative of demographic information available for AVMA and NAVTA Vet and VT populations in the US for age and sex distributions [16,35]. Furthermore, response rates were approximately equal for each occupational group. This would suggest comparisons between groups are not impacted by unequal sampling concerns.

Additional research is needed to further explore factors that may influence rabies vaccination practices and adherence to serological monitoring. Specifically the costs associated with pre-exposure vaccination and routine monitoring are likely to impact adherence levels, and are not covered in this analysis. Out-of-pocket costs for rabies pre-exposure vaccination exceeds $900, more than a week's net pay for those at or below the median household income level [36,37]. Pre-exposure vaccination may not be covered by a person's health insurance policy. One study found pre-exposure vaccination coverage by

employers occurred at about half the rate of coverage for PEP in the event of an exposure[18]. The findings in this analysis suggest additional training on rabies transmission routes and current recommendations for animal care workers is warranted. A study in West Virginia found that fewer than 30% of veterinary clinics had access to ACIP or NASPHV Rabies Compendium recommendations[18]. Methods to increase access and familiarity to these documents in the work setting should be considered. Similarly the factor with the greatest effect on adherence to rabies vaccination and serological monitoring were employer policies supporting these recommendations. Education outreach on rabies recommendations should include employers and include guidance on developing policies for vaccination and rabies serological monitoring programs. The veterinary standard precautions recommendations published by NASPHV covers employee vaccination monitoring, but these recommendations could be strengthened and reinforced in other rabies recommendation documents (i.e. ACIP and NASPHV Rabies Compendium).

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TABLES

Table 1. Demographic characteristics of study participants by occupational groups.

Category	Animal Control (n=445)		Veterinarian (n=375)		Veterinary Technician (n=1,357)		Wildlife Rehabber (n=157)		All (n = 2,334)		χ2	p-value
Age												
Mean	44.4		47.0		38.7		49.5		41.8		--	
95% CI	(43.3 - 45.6)		(45.6 - 48.3)		(38.1 - 39.2)		(47.4 - 51.7)		(41.3 - 42.3)			
Gender											261.5	<0.0001
Female	297	(66.7%)	294	(78.4%)	1285	(94.7%)	149	(94.9%)	2025	(86.8%)		
Male	148	(33.3%)	81	(21.6%)	72	(5.3%)	8	(5.1%)	309	(13.2%)		
Rabies Reservoir Region (%, n=2,328)												
Raccoon	179	(40.3%)	193	(51.6%)	679	(50.2%)	74	(47.1%)	1125	(48.3%)	28.2	<0.0001
Skunk / Fox / Mongoose	213	(48%)	132	(35.3%)	466	(34.4%)	60	(38.2%)	871	(37.4%)		
Bat / Rabies Free	52	(11.7%)	49	(13.1%)	208	(15.4%)	23	(14.6%)	332	(14.3%)		
Education (%; n=2,327)											2626.5	<0.0001
Highschool or Less	163	(36.8%)	0	(0%)	77	(5.7%)	22	(14.1%)	262	(11.3%)		
Associates/Bachelors	259	(58.5%)	4	(1.1%)	1207	(89.2%)	96	(61.5%)	1566	(67.3%)		
Veterinary Degree	0	(0%)	363	(96.8%)	2	(0.1%)	3	(1.9%)	368	(15.8%)		
Graduate (e.g. MS, PhD)	21	(4.7%)	8	(2.1%)	67	(5%)	35	(22.4%)	131	(5.6%)		
Household Income (%; n=2254)												
>=$50,000	258	(59.3%)	330	(93.5%)	677	(51.4%)	109	(73.2%)	1374	(61%)	2017.2	<0.0001
<$50,000	177	(40.7%)	23	(6.5%)	640	(48.6%)	40	(26.8%)	880	(39%)		
Years in Current Position												
Mean	10.3		15.1		9.6		12.1		10.8		--	
95% CI	(9.6 - 11.1)		(13.8 - 16.4)		(9.2 - 10.1)		(10.4 - 13.7)		(10.4 - 11.2)			
Previously vaccinated against rabies (n=2,318)												
Y	344	(78.5%)	367	(98.7%)	937	(69.3%)	122	(78.2%)	1770	(76.4%)	141.12	<0.0001
N	94	(21.5%)	5	(1.3%)	415	(30.7%)	34	(21.8%)	548	(23.6%)		

Table 2. Animal contact and rabies exposure risks among study participants by occupational groups.

Category	Animal Control (n=445)	Veterinarian (n=375)	Veterinary Technician (n=1,357)	Wildlife Rehabber (n=157)	All (n = 2,334)	χ2	p-value
Animals Work With (% respondents; n=2,310)							--
Companion Animals	441 (99.1%)	322 (85.9%)	1287 (94.8%)	35 (22.3%)	2085 (89.3%)		
Large Animals / Livestock	229 (51.5%)	95 (25.3%)	223 (16.4%)	10 (6.4%)	557 (23.9%)		
Exotic Pets / Birds / Reptiles	171 (38.4%)	97 (25.9%)	429 (31.6%)	22 (14%)	719 (30.8%)		
Birds	213 (47.9%)	74 (19.7%)	289 (21.3%)	87 (55.4%)	663 (28.4%)		
Reptiles	163 (36.6%)	58 (15.5%)	231 (17%)	58 (36.9%)	510 (21.9%)		
Feral Cats	358 (80.4%)	111 (29.6%)	436 (32.1%)	17 (10.8%)	922 (39.5%)		
Wildlife	235 (52.8%)	49 (13.1%)	158 (11.6%)	125 (79.6%)	567 (24.3%)		
Rabies Reservoirs	284 (63.8%)	33 (8.8%)	96 (7.1%)	109 (69.4%)	522 (22.4%)		
Animals Handled Annually (n=2,312)							
Geometric Mean	1006.9	2,314.0	2,415.6	972.2	1,908.0		--
95% CI	(913.1 - 1,110.4)	(2,086.0 - 2,567.0)	(2,308.3 - 2,527.8)	(782.3 - 1,208.3)	(1,829.3 - 1,990.0)		
Animal Bite Rate (n=2,302)							
Per 1 person year	0.45	1.00	0.66	1.66	0.77		
Geo. Mean (per 1,000 animals hand	0.07	0.05	0.06	0.20	0.07		--
95% CI	(0.05 - 0.10)	(0.04 - 0.07)	(0.05 - 0.07)	(0.11 - 0.36)	(0.06 - 0.08)		
Ever Handled a Suspect/Confirmed Rabid Animal (n=2,329)							
Yes	320 (71.9%)	226 (60.8%)	679 (50.1%)	79 (50.3%)	1304 (56%)	70.2	<0.0001
No / Don't Know	125 (28.1%)	146 (39.2%)	676 (49.9%)	78 (49.7%)	1025 (44%)		
Rate per 1 person year	3.68	0.67	1.21	0.76	1.50		
Suspect/Confirmed Rabid Animal Handling Rate (per 1,000 Animals handled; n = 1,268)*							
Geometric Mean	1.24	0.14	0.13	0.54	0.25		--
95% CI	(0.97 - 1.59)	(0.10 - 0.18)	(0.11 - 0.15)	(0.34 - 0.86)	(0.22 - 0.28)		
Potentially exposed to rabies during last contact with suspect/confirmed rabid animal (n=1,290)*							
Yes	60 (19.1%)	66 (29.3%)	194 (28.8%)	23 (29.5%)	343 (26.6%)	11.93	0.007
No / Don't Know	254 (80.9%)	159 (70.7%)	479 (71.2%)	55 (70.5%)	947 (73.4%)		
In current position, received rabies PEP due to potential exposure (n=1,295)*							
Yes	68 (21.7%)	56 (24.8%)	110 (16.2%)	37 (47.4%)	271 (20.9%)	44.21	<0.0001
No / Don't Know	246 (78.3%)	170 (75.2%)	567 (83.8%)	41 (52.6%)	1024 (79.1%)		
Rate per 100 person years	1.47	0.99	0.84	1.95	1.07		
Source of Potential Rabies Exposure for which PEP received (n=269)							--
Cat	20 (29.4%)	24 (42.9%)	56 (50.9%)	1 (2.7%)	101 (37.3%)		
Dog	5 (7.4%)	6 (10.7%)	22 (20%)	0 (0%)	33 (12.2%)		
Livestock	0 (0%)	13 (23.2%)	8 (7.3%)	1 (2.7%)	22 (8.1%)		
Bat	9 (13.2%)	2 (3.6%)	6 (5.5%)	17 (45.9%)	34 (12.5%)		
Fox	5 (7.4%)	0 (0%)	1 (0.9%)	2 (5.4%)	8 (3%)		
Raccoon	16 (23.5%)	4 (7.1%)	10 (9.1%)	13 (35.1%)	43 (15.9%)		
Skunk	7 (10.3%)	4 (7.1%)	2 (1.8%)	0 (0%)	13 (4.8%)		
Other / multiple species	5 (7.4%)	2 (3.6%)	5 (4.5%)	3 (8.1%)	15 (5.5%)		

*Restricted to persons reportign a history of ever handling a suspect/confirmed Rabid Animal

Table 3. Knowledge of rabies vaccination recommendations by occupational groups

Category	Animal Control (n=445)		Veterinarian (n=375)		Veterinary Technician (n=1,357)		Wildlife Rehabber (n=157)		Total (n=2,334)		χ2	p-value
Rabies Infectious Materials (% responding)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)		--
Saliva	431	(96.9%)	374	(99.7%)	1334	(98.3%)	154	(98.1%)	2293	(98.2%)		
Nervous Tissue	248	(55.7%)	325	(86.7%)	959	(70.7%)	88	(56.1%)	1620	(69.4%)		
Urine	70	(15.7%)	68	(18.1%)	319	(23.5%)	34	(21.7%)	491	(21%)		
Blood	200	(44.9%)	149	(39.7%)	728	(53.6%)	68	(43.3%)	1145	(49.1%)		
Don't Know	5	(1.1%)	0	(0%)	16	(1.2%)	2	(1.3%)	23	(1%)		
Number of vaccine doses for pre-exposure vaccination (n=2,294)											44.04	<0.0001
Less than 3 doses	40	(9.1%)	67	(18.1%)	226	(16.9%)	7	(4.8%)	340	(14.8%)		
3 doses	353	(80.2%)	290	(78.2%)	1029	(76.9%)	125	(86.2%)	1797	(78.3%)		
More than 3 doses	47	(10.7%)	14	(3.8%)	83	(6.2%)	13	(9%)	157	(6.8%)		
What is administered after a rabies exposure for a person who is previously vaccinated against rabies (n=2,331)											140.7	<0.0001
2 doses of vaccine	83	(18.7%)	132	(35.2%)	205	(15.1%)	55	(35.3%)	475	(20.4%)		
4 doses of vaccine	18	(4%)	9	(2.4%)	27	(2%)	3	(1.9%)	57	(2.4%)		
Rabies immunglobulin + vaccine	207	(46.5%)	162	(43.2%)	582	(43%)	67	(42.9%)	1018	(43.7%)		
Nothing	24	(5.4%)	4	(1.1%)	52	(3.8%)	4	(2.6%)	84	(3.6%)		
Don't know	113	(25.4%)	68	(18.1%)	489	(36.1%)	27	(17.3%)	697	(29.9%)		
How often should a previouly vaccinated person have their titer checked if they work with animals in a rabies endemic area?											72.12	<0.0001
Every 5 years	84	(18.9%)	96	(25.6%)	298	(22%)	11	(7%)	489	(21%)		
Every 2 years	200	(44.9%)	171	(45.6%)	515	(38%)	81	(51.6%)	967	(41.4%)		
Every year	82	(18.4%)	52	(13.9%)	284	(20.9%)	44	(28%)	462	(19.8%)		
Every 6 months	12	(2.7%)	2	(0.5%)	30	(2.2%)	10	(6.4%)	54	(2.3%)		
Never	2	(0.4%)	1	(0.3%)	2	(0.1%)	0	(0%)	5	(0.2%)		
Don't know	65	(14.6%)	53	(14.1%)	228	(16.8%)	11	(7%)	357	(15.3%)		
According to US recommendations a person should receive a rabies vaccine booster when their titer drops below this level											117.9	<0.0001
Correct to ACIP recommendations	40	(9%)	45	(12%)	99	(7.3%)	30	(19.1%)	214	(9.2%)		
Correct to WHO recommendations	87	(19.6%)	134	(35.7%)	210	(15.5%)	35	(22.3%)	466	(20%)		
Incorrect response	16	(3.6%)	11	(2.9%)	39	(2.9%)	5	(3.2%)	71	(3%)		
Don't Know	302	(67.9%)	185	(49.3%)	1009	(74.4%)	87	(55.4%)	1583	(67.8%)		
Does your employer have a policy requiring you to be vaccinated against rabies? (n=2,298)											93.18	<0.0001
Yes	169	(38.9%)	83	(22.6%)	245	(18.2%)	58	(37.9%)	555	(24.2%)		
No / Don't know	266	(61.1%)	284	(77.4%)	1098	(81.8%)	95	(62.1%)	1743	(75.8%)		
Does your employer have a policy requring you to have your rabies titer checked periodically? (n=2,296)											68.14	<0.0001
Yes	112	(25.7%)	47	(12.8%)	194	(14.5%)	54	(35.5%)	407	(17.7%)		
No / Don't know	323	(74.3%)	320	(87.2%)	1148	(85.5%)	98	(64.5%)	1889	(82.3%)		

Table 4. Univariate analysis of factors associated with rabies vaccination and current serologic monitoring status.

	Rabies Vaccination					Current Titer				
Category	Y (n=1,770)	N (n=548)	OR	95% CI	p-value	Y (n=613)	N (n=392)	OR	95% CI	p-value
Age					<0.0001					0.0011
6 - 31 Years	362	207	ref	-		104	37	ref	-	
32 - 50 Years	853	235	2.08	(1.66 - 2.59)		322	206	0.56	(0.36 - 0.84)	
51 - 78 Years	555	106	2.99	(2.29 - 3.92)		187	149	0.45	(0.29 - 0.69)	
Gender					0.0123					0.385
Female	1519	493	ref	-		540	338	ref	-	
Male	251	55	0.68	(0.49 - 0.91)		73	54	0.85	(0.58 - 1.24)	
Rabies Region					<0.0001					0.0797
Raccoon	938	179	3.78	(2.88 - 4.97)		404	237	1.26	(0.97 - 1.64)	
Skunk / Fox / Mongoose	637	230	2	(1.53 - 2.61)		209	155	ref	-	
Bat / Rabies Free	187	135	ref	-		n/a	n/a	n/a	n/a	
Education					<0.0001					0.1189
Highschool or Less	182	78	ref	-		60	35	ref	-	
Associates/Bachelors	1116	440	1.09	(0.81 - 1.44)		368	221	0.97	(0.62 - 1.52)	
Veterinary Degree	360	5	30.71	(13.07 - 87.23)		139	114	0.71	(0.44 - 1.15)	
Graduate (e.g. MS, PhD)	106	24	1.89	(1.14 - 3.21)		44	21	1.22	(0.63 - 2.40)	
Household Income					<0.0001					0.0111
<$50,000	672	285	ref	-		399	283	ref	-	
≥$50,000	1098	263	1.77	(1.45 - 2.16)		191	93	1.46	(1.09 - 1.95)	
Years in Current Position					<0.0001					0.0287
0-3 years	485	211	ref	-		127	74	ref	-	
4-8 years	299	135	0.96	(0.74 - 1.25)		112	51	1.28	(0.82 - 1.99)	
9-16 years	481	118	1.77	(1.37 - 2.30)		181	112	0.94	(0.65 - 1.36)	
>16	505	84	2.61	(1.98 - 3.48)		193	155	0.72	(0.51 - 1.04)	
Occupation Category					<0.0001					0.0137
Veterinarian	367	5	32.51	(13.35 - 79.16)		143	116	0.82	(0.61 - 1.11)	
Veterinary Technician	937	415	ref	-		300	200	ref	-	
Animal Control	344	94	1.62	(1.26 - 2.09)		120	54	1.48	(1.03 - 2.15)	
Wildlife Rehabilitator	122	34	1.59	(1.08 - 2.39)		50	22	1.51	(0.89 - 2.62)	
Work with rabies reservoir species					<0.0001					0.0032
Yes	440	77	2.04	(1.56 - 2.65)		180	83	1.56	(1.16 - 2.12)	
No	1311	467	ref	-		424	306	ref	-	
Animals Handled Annually*					0.013					0.012
52 - 1040	388	91	ref	-		146	70	ref	-	
1041 - 2600	799	252	0.74	(0.57 - 0.97)		278	171	0.78	(0.55 - 1.10)	
2601 - 3900	193	56	0.81	(0.56 - 1.18)		72	45	0.77	(0.48 - 1.23)	
>3900	390	149	0.61	(0.46 - 0.82)		117	106	0.53	(0.36 - 0.78)	
Animal Bite Rate (per 1,000 Animals handled)*					0.121					0.089
<0.05	496	143	ref	-		170	124	ref	-	
0.05 - 0.45	844	248	0.98	(0.78 - 1.24)		296	196	1.1	(0.82 - 1.48)	
>0.45	430	157	0.79	(0.61 - 1.02)		147	72	1.49	(1.03 - 2.15)	
Ever Handled a Suspect Rabid Animal*					<0.0001					0.0035
Y	1070	219	2.3	(1.89 - 2.80)		441	248	1.5	(1.14 - 1.96)	
N / Don't Know	697	328	ref	-		171	144	ref	-	
Risk Group					0.389					0.0475
4 (least risk averse)	152	38	ref	-		58	20	ref	-	
3	185	63	0.73	(0.46 - 1.16)		54	41	0.46	(0.23 - 0.87)	
2	294	78	0.94	(0.61 - 1.95)		109	60	0.63	(0.34 - 1.14)	
1 (most risk averse)	1022	318	0.8	(0.54 - 1.16)		362	246	0.51	(0.29 - 0.86)	
Employer policy requiring:	rabies vaccination				<0.0001	periodic titer checks				<0.0001
Yes	545	10	24.2	(12.84 - 45.61)		276	51	5.47	(3.93 - 7.70)	
No / Don't Know	1207	536	ref	-		335	339	ref	-	

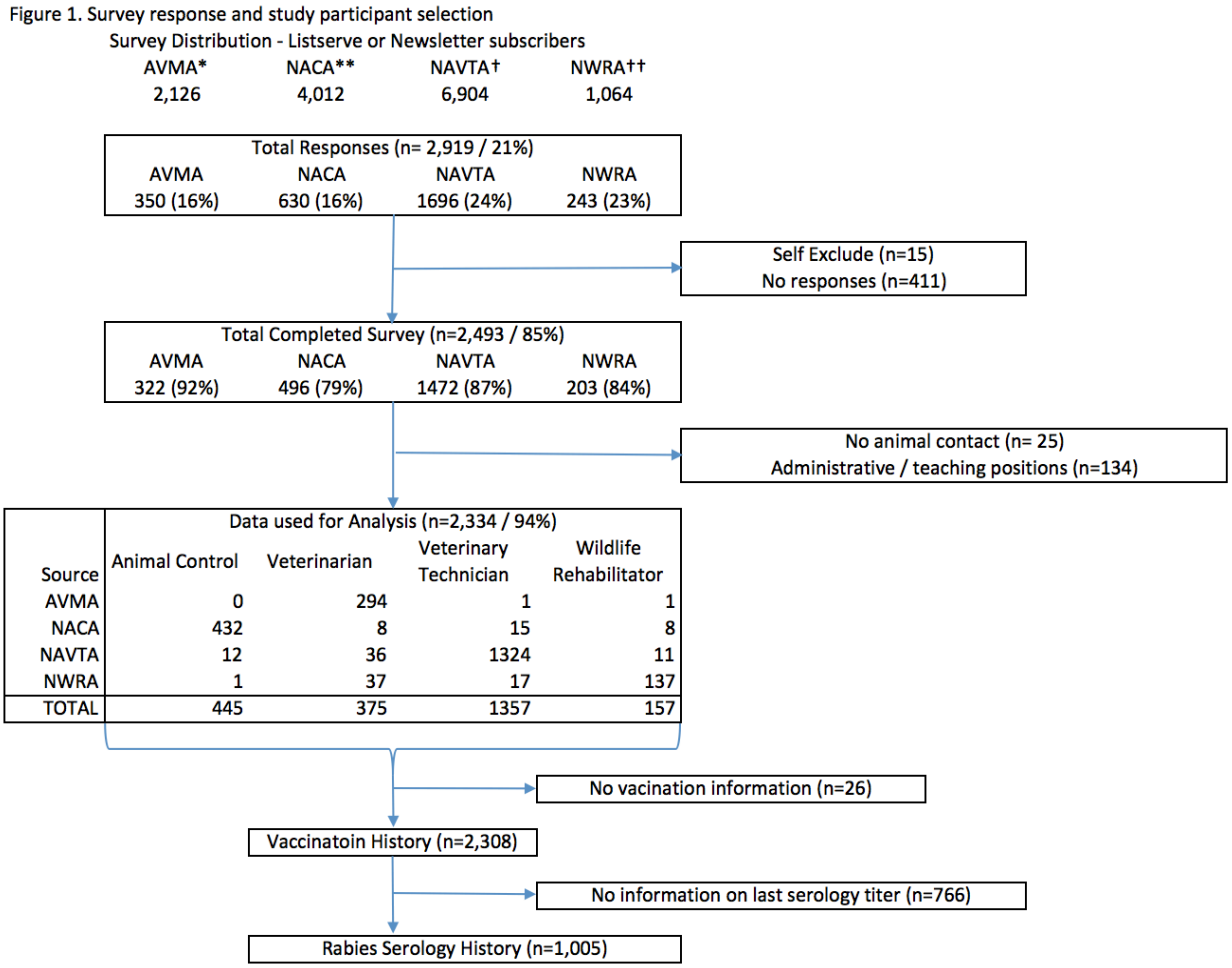
Table 5. Multivariate analysis of factors associated with a) rabies vaccination and b) current serological monitoring status.

Parameter	OR	95% CI
A) Vaccination		
Rabies Reservoir Region (ref. Bat/Free)		
Raccoon	3.17	(2.30 - 4.36)
Skunks/Fox/Mongoose	1.70	(1.24 - 2.34)
Education (ref. <= Highschool)		
Associates/Bachelors	1.81	(1.24 - 2.65)
DVM	1.98	(0.22 - 17.63)
Graduate	2.77	(1.48 - 5.18)
Years in Current Position	1.04	(1.02 - 1.05)
Occupational Group (ref. vet tech)		
Animal Control	1.51	(1.07 - 2.12)
Veterinarian	33.24	(3.18 - 347.65)
Wildlife Rehabilitator	1.10	(0.68 - 1.80)
Handled Suspect Rabid Animal	1.64	(1.30 - 2.08)
Risk Aversion Group (1-4)	1.07	(0.95 - 1.20)
Employer policy requring vaccination	32.16	(14.18 - 72.94)
B) Current Serological Monitoring		
Age	0.98	(0.96 - 0.99)
Raccoon reservoir region (refs. Skunk/Fox region, Veterinary Technicians)		
Animal Control Workers	1.73	(0.82 - 3.67)
Veterinarians	1.50	(0.85 - 2.64)
Wildlife rehabilitators	0.13	(0.04 - 0.47)
Income (ref. <$50,000)	1.52	(1.06 - 2.16)
Occupational Groups (refs. Raccoon region, Veterinary Technician)		
Animal Control Workers	1.21	(0.64 - 2.28)
Veterinarian	1.36	(0.88 - 2.12)
Wildlife rehabilitators	0.37	(0.16 - 0.89)
Number of Animals Handled Annually*	0.80	(0.68 - 0.94)
Handled suspect rabid animal	1.47	(1.07 - 2.02)
Risk Aversion Group	1.10	(0.94 - 1.28)
Employer policy requiring titer check	6.14	(4.18 - 9.02)

*Logarithmically transformed

FIGURES

Figure 1. Study Response and Study Participant Selection



CHAPTER 4: IMMUNE RESPONSE TO PRIMARY RABIES PRE-EXPOSURE VACCINATION AND BOOSTER VACCINATION: A SYSTEMATIC REVIEW AND META-ANALYSIS*

* Blanton, Jesse D. To be submitted to Vaccine

ABSTRACT

Objective: A systematic review and meta-analysis was conducted to synthesize the current evidence available in the literature with a focus on rabies virus neutralizing antibody response (as measured by seroconversion rates (SCR) and geometric mean titers (GMT)) generated by different vaccines, administration routes, doses, and schedules.

Design: The MEDLINE, EMBASE, Cochrane Library, and WHO Index Medicus database were systematically searched. After a primary screening, 120 articles were identified for further review, of which 51 (42.5%) met inclusion criteria. Critical review and data collection was conducted by two reviewers and entered into a database for analysis.

Results: The intramuscular (IM) vaccine administration route showed relatively little variation in primary SCRs from different administration routes and schedules compared to the intradermal (ID) vaccine route. In general, the ID route had slightly lower estimated SCRs after primary vaccination. However, all study cohort subjects responded to a booster vaccination or simulated postexposure prophylaxis regardless of vaccine, route, schedule, length of time since primary vaccination, or titer at time of booster.

Conclusions: Primary SCRs were robust when administered by the IM route regardless of deviations from current recommendations. This provides some evidence that shorter administration routes could be considered for future recommendations. Furthermore, while ID vaccination does not appear to be inferior to IM vaccination routes, it appears

careful consideration of the vaccine and route is more warranted. Multi-site ID regimens may be preferred to ensure complete SCRs maintain higher GMTs for a longer duration of time. Furthermore given the robust response to booster vaccination, future evaluations should examine the objectives of routine serological monitoring for risk groups and if currently recommended frequencies are cost effective or necessary.

INTRODUCTION

Rabies remains a global public health threat with more than 60,000 human rabies cases estimated to occur annually and an additional 30 million exposures resulting in postexposure prophylaxis (PEP) [1]. The majority of this burden remains in developing countries where canine rabies is endemic. However, exposure to *Lyssaviruses* (the etiologic agent of rabies) from bats and other wildlife remains a threat globally. In the United States, the national incidence of rabies exposures resulting in PEP is estimated around 8.5 / 100,000 persons, however, the risk of exposure among persons who have increased contact with animals (either due to occupational or leisure activities) has been reported as high as 1,000/ 100,000 person years [2]. Based on the risk of higher exposure, the World Health Organization (WHO) and US Advisory Committee on Immunization Practices (ACIP) recommend pre-exposure vaccination (preEV). While the cost effectiveness of utilizing preEV among larger segments of the population at increased risk (e.g. children in canine rabies endemic countries) is debated, its recommended use among high-risk occupational groups has been in place for more than 40 years [3,4].

PreEV consists of three doses of rabies vaccine administered on days 0, 7, and 21 or 28 [5]. While ACIP recommendations stipulate intramuscular (IM) administration, WHO includes both IM and intradermal (ID) routes in their recommendations [3,5]. Pre-

exposure vaccination does not preclude the need for PEP, but does simplify the intervention by removing the need for rabies immunoglobulin and reducing the recommended series to two vaccine doses (administered on days 0 and 3). Current WHO recommendations stipulate vaccines must contain a potency of 2.5IU to be used for rabies preEV or PEP, but in practice may be much higher [3,6]. Many cell culture vaccines are available globally that meet this qualification and have been reviewed by WHO, though few have gone through the pre-qualification process [7,8]. In general, these vaccines are classified by the cell culture medium on which they are produced: Human Diploid Cell Vaccines (HDCV), Purified Chick Embryo Cell Vaccine (PCECV), Purified Vero Cell Rabies Vaccine (PVRV), Primary Hamster Kidney Cell Vaccine (PHKCV), and Purified Duck Embryo Vaccine (PDEV) [9].

Confirmation of sero-conversion is not routinely recommended after rabies vaccination [5]. While higher titers have been roughly correlated with an increased probability of protection against rabies infection, no minimum protective rabies virus neutralizing antibody (RVNA) titer has been identified [5]. However, periodic serologic monitoring is recommended for persons with high exposure risk to ensure a specified minimal antibody level is maintained. This is to ostensibly protect against unrecognized exposures among high-risk populations, though this is not well evaluated [5]. No human rabies cases have been reported among previously vaccinated persons who received a booster after a potential exposure, and only one death has been reported in a previously vaccinated person who failed to receive a booster [10,11]. Current serologic monitoring guidelines in the US recommend monitoring every 6 months for “continuous” high-risk groups (e.g. rabies laboratory workers) and every 2 years for “frequent” risk groups (e.g. animal health workers in rabies endemic areas). For persons in these risk groups a

vaccine booster is recommended when their titer falls below complete neutralization at a serum dilution of 1:5 (approximately 0.1-0.2 IU/mL) using the Rapid Fluorescent Focus Inhibition Test (RFFIT) [5]. Alternatively, WHO uses a more conservative cut-off for evaluating titers, recommending boosters when a person's titer drops below 0.5 IU/mL (approximately complete neutralization at a serum dilution of 1:25) [3]. Regardless, serologic monitoring by virus neutralization tests remains, at best, a surrogate measurement to determine the overall anti-rabies immune status and probability of an anamnestic response in the event of a rabies exposure [5,12].

Several studies have evaluated the long-term persistence of rabies neutralizing antibodies since the licensure of modern cell culture based rabies vaccines. However, few studies have monitored RVNA response longer than 2-3 years, or have involved routes of administration or schedules that may not reflect current recommendations [13-15]. Evaluation of the current evidence related to primary RVNA response and seroconversion after preEV as well as long-term duration of immunity is important for the process of updating current recommendations related to human rabies vaccination. In particular, recommendations related to the type of vaccine, route of vaccine administration, number of vaccine doses, and schedule of administration, may impact individual response and are components easily addressed by current recommendations. A thorough review of the literature to synthesize existing evidence may identify changes to the current recommendations for preEV and serological monitoring.

METHODS

A protocol based on the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines was developed [16]. Keywords were developed

from representative studies identified by subject matter experts. A combination of medical subject headings (MeSH) terms and text words related to rabies pre-exposure vaccination were identified. The final search term used the key words ("rabies" OR "rabies vaccine"). Additional Boolean operators included "antibodies" AND "human" AND ("preexposure" OR "pre-exposure"). The search was conducted in four electronic databases (MEDLINE, Embase, Cochrane Library, and at regional WHO Index Medicus sites). Studies published from January 1, 1965 to December 31, 2015 were collected. Search results for each database were exported to a master database and consolidated to a list of unique studies.

Two reviewers conducted an initial screen of the results based on title and abstract analysis for each manuscript. The population, intervention, comparison, and outcome (PICO) criteria were determined to focus inclusion criteria. The population was identified as persons at risk of rabies exposure. The intervention was rabies pre-exposure vaccination using a WHO reviewed vaccine. Comparisons were between antibody response over time following completion of vaccination and differences in response between persons receiving different vaccination routes and schedules. The outcome of interest was neutralizing antibodies as determined by the RFFIT.

Studies excluded from the analysis included those from non-peer reviewed sources, those focusing on vaccination recommendations or reviews, studies evaluating only vaccines not reviewed by WHO[9], and studies evaluating only immunocompromised populations. Following this initial screening, studies were further evaluated on three eligibility requirements: 1) subjects received stated pre-exposure vaccination consisting of 1-3 vaccine doses over ≤1 year, 2) immune response was measured using RFFIT, and

3) findings reported as geometric mean titers with variance or serocoversion rate (SCR) at a stated RVNA cut-off limit.

A panel of 14 reviewers received a one-hour training session consisting of the review objectives, overview of collection tools, and copies of other resources and reference material that might aid the review process. Two reviewers evaluated each study for inclusion and exclusion criteria. A standardized data collection tool was provided to perform a structured review and data collection. The tool collected information on exclusion and inclusion criteria, basic study demographics (e.g. study methodology, sample size, intervention), and data collection on vaccination schedule and immune response over the course of the study. Internal and external validity was evaluated as 'good', 'fair', or 'poor' as outlined by the US Preventive Services Taskforce guidelines[17]. Where studies included interventions outside the scope of this review (e.g. evaluated both preEV and postexposure prophylaxis models) only the relevant intervention data was collected. Where applicable kappa scores were calculated to evaluate concordance between reviewers[18].

Study data was entered into an Access database to describe qualitative trends of the selected studies. Specifically, analysis was performed on the study populations, vaccination protocols, follow-up time, and response to post vaccination boosters. Meta-analysis of study cohort GMTs and SCRs was conducted using Open Meta Analyst [19]. This analysis included sub-group analysis by vaccine type, route of administration, vaccination schedule, and follow-up period. Heterogeneity using the Q method and I2 test was evaluated and where significant ($P<0.1$) a random effects model using the DerSimonian and Laird methodology was used for subsequent analysis [20]. An additional meta-analysis based on reported maintenance of SCRs over time was

conducted to estimate a summary survival curve of seroconversion as previously described [21]. This method requires survival measurements at all interval time periods. In some cases this required missing data from cohorts to be assumed (i.e. survival rate constant before and after the missing time period) or in rare cases interpolated as a linear association between the two known measurements. To generate the summary survival curve the package 'MetaSurv' in R was utilized [22,23].

RESULTS

Literature Search

After primary screening, 120 studies were selected from the databases for critical review. Of these, 51 (42.5%) articles were eligible for inclusion in the systematic review (Figure 1). Agreement between reviewers was high (kappa = 0.62; 95% Confidence Interval 0.48 – 0.76). Only 23 studies required reconciliation due to discordance between reviewers and all but three of these were ultimately rejected after a third review. Of the 51 accepted studies, 41 (80.4%) contained sufficient information for further meta-analysis. Accepted studies were published between 1978 and 2014. A total of 6,170 participants were included in across 112 cohorts (Table 1)[13,15,24-72].

Study Characteristics

The majority (n=28, 54.9%) of studies were randomized trials. Another nine (17.6%) were non-randomized trials, five (9.9%) were cohort studies (3 prospective and 2 retrospective), and nine (17.6%) were case series. Internal validity assessments by the reviewers found 26 (50.9%) studies had good internal validity, 24 (47.0%) had fair internal validity, and 1 (2%) study was rated poor [42]. This study was rated poor because

it did not provide clear descriptions of subject selection process and intervention cohorts were not comparable. The decision to retain the study was based on availability of GMT data from one cohort that appeared to be accurately collected and determined to potentially be of value for the meta-analysis. External validity assessments by the reviewers found 26 (50.9%) studies had good external validity, 22 (43.1%) had fair external validity, and 3 (5.8%) were rated as poor [28,49,53]. These three studies evaluated preEV in young children using a pediatric vaccination schedule, which is not currently a recommended strategy for preEV. However, these studies were retained because they represented some of the few studies evaluating preEV and RVNA response among children.

Studies were conducted in 15 countries across 6 regions: South East Asia (17 studies), North America (16), Europe (9), South Asia (6), South America (2), and Africa (1). The majority of studies were conducted on at at-risk or veterinary school populations (n=28, 54.9%). Additional studies were conducted on the general community (typically in canine rabies endemic countries) populations (27.4%), children (13.7%), and international travelers (3.9%). Where provided, the age range for included studies was between 5 and 75 years old. Among the seven studies evaluating preEV in children, three recruited infants primarily <1 year old, the other studies recruited slightly older child population up to 12 years old. Nearly all (n=44, 86%) studies evaluated SCR based on the WHO recommended cut-off of ≥0.5IU/mL, (11 studies evaluated at the ACIP recommended cut-off of ≥0.11IU/mL). Subsequently the WHO cut-off was used for all further analysis of SCRs.

Vaccine Type

Twenty-five studies evaluated responses to HDCV, 23 to PVRV, 13 to PCEC, and 3 to other vaccines (i.e. bovine or hamster kidney cell, and PDEV). However, only 18 (35%) studies were designed to directly compare RVNA response between types of vaccines. Of these, 13 (72%) studies evaluated one or more vaccines against HDCV and 5 (28%) studies evaluated one or more vaccines against PVRV (Table 1). Vaccines evaluated against HDCV included other formulations of HDCV [13,71], PVRV [24,47,50,52,55,57,63], PCEC [13,15,32,63], PDEV [70], and others (fetal bovine kidney vaccine and PDEV) [63]. Vaccines evaluated against PVRV included other formulations of PVRV [27,31,34,39] and PCEC [44]. Ten (55%) of the studies found no significant difference in GMTs between vaccines evaluated. Three of the four studies evaluating HDCV and PCEC found a significantly higher primary RVNA response to HDCV within several weeks of completing the vaccination series [13,32,63]. However, two of these studies found significantly higher GMTs for cohorts that received PCEC when compared at 1-2 years post vaccination [13,32]. The third study did not monitor response rates beyond 90 days [63]. Significant differences in RVNA response were reported in three (43%) of the studies evaluating HDCV and PVRV vaccines. Of these studies, two reported a significantly higher RVNA response at 60 days and 2 years among cohorts administered PVRV [24,55]. However, a third study, found a significantly lower GMT response among persons receiving PVRV at 1 year [50]. However, this study evaluated a more purified PVRV product and a younger cohort than used in other studies. The HDCV vaccine was reported to produce significantly higher RVNA responses compared to PDEV and FBKC vaccines.

In addition to evaluating differences in RVNA response between rabies vaccines, three studies evaluated the impact of co-administering rabies vaccine with childhood immunization schedules against diphtheria, tetanus, pertussis, and polio (DTP-IPV) in

pediatric populations [28,49,53]. No significant interference from rabies vaccination on the response to DTP-IPV was reported and all subjects receiving rabies vaccine seroconverted after completing 2-3 doses of rabies vaccine over the course of 60 days.

Administration Route

The majority of studies (40/51; 78%) utilized an intramuscular route to administer rabies vaccine to subjects. Twenty-five (49%) studies utilized an intradermal route to administer vaccine, including four that evaluated multi-site (i.e. administration of two 0.1mL doses at different sites on each day of the schedule) administration routes and six studies that administered ID using PVRV which is packaged at 0.5mL resulting in a more concentrated dose by the ID route. Four studies administered vaccine using a sub-cutaneous route.

Among the selected studies, only 16 were designed to directly evaluate the differences in RVNA response between different vaccine administration routes. The IM route was evaluated in all 16 studies, 14 included a cohort of subjects administered vaccine via the ID route [25,31,36,38,41,45 13,49,51,58,62,66,69,72], 4 studies included the 2-site ID route [25,35,36,43], and 2 studies included the subcutaneous (SC) route [62,69]. All studies evaluating IM and ID (at the commonly administered 1.0mL and 0.1mL dosage respectively), found a significantly higher GMT and SCR over time among persons receiving vaccine via the IM route. A single study compared IM and ID administration routes at multiple vaccine dilutions [72]. In this study, ID administration produce a higher GMT than IM administration at 90 days post vaccination when each route administered equal volume doses. Similarly, studies evaluating 2-site ID administration routes routinely reported a higher GMT compared to single-site ID administration [25,36], but

lower response compared to full-dose IM administration routes [25,35,36,43]. The SC route (at 0.1mL dose) was reported to produce lower RVNA responses at 90 days and 1-year post vaccination compared to both the ID and IM route.

Vaccination Schedule

Forty-three (84%) studies administered rabies vaccine according to the WHO and ACIP recommended schedule (i.e. on days 0, 7, 21 or 28). Three (6%) studies administered three total doses over an expedited 7-day schedule. Twelve (23%) studies administered two doses of vaccine; one over a single day, one over seven-days, one over 21-days, and five over 28-days. Three (6%) studies administered only a single dose of vaccine. Three studies evaluated addition of rabies vaccine to a childhood vaccination schedule and administered two doses of vaccine over 60 days or three doses over 120 days.

In total, eight studies were designed so as to be able to directly compare RVNA response between different vaccine administration schedules [26,35,36,43,45,49,52,70]. However, only six studies compared multiple administration schedules using the same route. All compared alternate schedules to the current recommendations (4 by ID route, 1 by IM route, and 1 by SC route). The primary SCR was nearly 7 times lower for persons receiving a single dose of vaccine (Table 2). Summary SCRs for recipients of two doses were higher compared to recipients of a full 3-dose schedule. Two studies evaluated expedited schedules (i.e. days 0, 3, and 7) compared to the standard recommended schedule; both studies utilized a 2-site ID administration route [43,45]. These studies reported somewhat higher geometric mean titers among subjects receiving the 7-day schedule, but no significant difference from the recommended schedule or by the IM route was reported. Three studies administered vaccine over a 60-day period as part of a

childhood immunization schedule, all of which reported 100% sero-conversion to the rabies vaccine by day 90.

Primary Response and Duration of Immunity

Among the 112 study cohorts in the selected studies that met inclusion criteria, the median follow-up time from start of vaccination to last RVNA titer before any booster doses were administered was 360 days (range: 28 days – 6 years). Nearly all study cohorts completed primary follow-up at one of the following periods: 45 days, 90 days, 365 days, or 730 days (Figure 2).

A total of 97 cohorts reported measurements of the primary SCR between 28-90 days after starting vaccination. Seroconversion after primary vaccination was reported among nearly all subjects with the exception of 19 cohorts from 11 studies [25,26,34,36,38,42,51 59,62,69,72]. A high SCR between 90-99% was achieved for 13 of these 19 cohorts, seven of which may have achieved 100% seroconversion if additional follow-up had been available. Six cohorts reported much lower primary SCRs, between 40-89%. No significant difference in primary SCR was observed between vaccines types administered by the IM route. However, primary SCRs were lower for all vaccine types by the ID route. The estimated primary SCR among subjects that received PCEC vaccine by the ID route was significantly lower than HDCV by the same route and significantly lower than PCEC vaccine received by the IM route (Table 2, Sup. Figure 1). Because PCEC vaccine was over represented among cohorts receiving ID administration, analysis was stratified to compare cohorts excluding these vaccines. Exclusion of PCEC vaccine had little effect on primary SCRs by administration route with the exception of the ID group, which was about 2% higher. However, less heterogeneity was observed across all

groups (with the exception of ID with PVRV vaccine) when PCEC vaccines were excluded. Two outlier studies appeared to account for the majority of the variation in the ID group [25,36]. However, no unique attributes could be identified about these studies that might account for the lower GMTs and SCRs reported. No significant difference was observed between administration routes; however, lower SCRs were reported among ID routes, particularly those that administered multiple functional doses (i.e. 2-site and ID with PVRV) (Table 2, Sup. Figure 2). The estimated primary SCR for 1-dose preEV schedules was significantly lower than 2- and 3- dose series (Table 2, Sup. Figure 3). No significant differences were observed between 7-, 21-, or 28-day schedules when the IM route was used; however, the 7-day schedule SCR was 4.5% lower than the other schedules (Table 2, Sup. Figure 4). The 21-day schedule had a higher estimated SCR compared to 28-day when vaccine was administered via the ID route.

A total of 52 cohorts measured RVNAs at day 365. The overall SCR was approximately 50% at one year. Heterogeneity was much higher within all sub-groups measured at 1-year compared to primary SCRs (Table 3). The estimated SCRs for PCEC vaccine was lower compared other vaccines regardless of the administration route (Sup. Figure 5). Similar to observations of primary SCR, administration of PCEC vaccine by the ID route resulted in lower SCRs (Table 3, Sup. Figure 6). Estimated SCR at one year was higher for ID routes administered by the PVRV route compared to both the IM and standard ID route. Estimated SCRs were highest for preEV schedules that administered 3-doses regardless of route (Table 3, Sup Figure 7). Schedules that administered 2-doses of vaccine were slightly lower compared to the 3-dose schedules by the IM route; however, a single dose resulted in significantly lower estimated SCRs. Regardless of the route, the 7-day schedule resulted in higher estimated SCR compared to the other

schedule lengths (Table 3, Sup. Figure 8). Otherwise, not significant difference in estimated SCR was observed between the 21 and 28 day schedules.

Analysis of total survival curves by administration route for 54 study cohorts (34 by IM and 20 by ID) also found relatively high variance between cohorts even when restricted to groups receiving 3 doses of vaccine. The estimated summary median survival time for persons receiving preEV by the ID route was 584 days, compared to >810 days for persons administered vaccine by the IM route (Figure 3).

The number of studies that reported sufficient data including variance on GMTs was limited (n = 52). The majority (43, 67%) of these cohorts were administered preEV by the standard IM ACIP recommended schedule making sub-analysis by route, dose, and schedule difficult. Sub-analysis of these cohort GMTs over time (days 28, 45, and 365) found significant heterogeneity at each time period (Figure 3). Additional sub-analysis by vaccine type and schedule duration at each measurement time did not significantly decrease the observed heterogeneity. Overall the estimated GMT for persons receiving 3-doses of vaccine by the IM route at 28 days was 22.2IU/mL (95% CI: 18.05 – 27.3 IU/mL). This dropped to 17.5IU/mL (95% CI: 14.5 – 21.2 IU/mL) by day 45, and to 1.71 IU/mL (95% CI: 1.21 – 2.41 IU/mL) 1-year after starting vaccination.

Response to booster vaccination

More than 2,600 subjects in 61 study cohorts from 18 studies received a booster dose or simulated PEP (i.e. doses on days 0 and 3) between 84 days and 11 years after primary vaccination (Table 4). At seven days post-booster the median increase observed among cohorts was 23 fold higher than the cohorts GMT (range: 1.5 – 141.6). Only five cohorts from two studies reported less than a 4-fold increase in GMT by 7 days post-booster

[25,54]. Four of these cohorts reported anamnestic responses (>4-fold increase) by day 14 post-booster [25]. Three cohorts never reported greater than a 4-fold increase in GMT; however, all three had relatively high pre-booster GMTs [54,62,65]. Larger increases in cohort GMTs were observed among cohorts with lower pre-booster GMTs (Figure 4). Cohorts receiving primary vaccination by the intramuscular route reported a higher median GMT seven days after receiving a booster compared to those vaccinated by the ID route. However, median GMTs among cohorts vaccinated by the IM and 2-site ID route were approximately equivalent by day 14 post-booster.

Across all study cohorts only one subject was reported that did not respond to a booster dose [34]. This subject was later diagnosed with lymphoma. Otherwise all subjects seroconverted (>0.5IU/mL) by 14 days post-booster regardless of primary vaccination methods, time since vaccination, or titer at booster.

LIMITATIONS

All SCRs and GMTs provided in the selected papers for this review were based on the RFFIT, which is a cell culture-based assay involving live rabies virus. Performance of this test can be susceptible to laboratory conditions operator training. No recognized national or global standard protocol for the RFFIT is available nor is proficiency known for the laboratories conducting these studies [73]. Restricting our analysis to only studies that used the RFFIT and reported in IU/mL (which requires a standard reference serum to be run with the samples) reduces this variation, but some non-systematic bias likely persists across studies and over time [74]. The use of a cut-off of 0.5IU/mL for determining SCR is a conservative approach to address this issue and has been recommended as evidence of true RVNA in a sample [11].

Some sub-analysis resulted in relatively small numbers of cohorts to estimate summary GMTs and SCRs. This may have resulted in underestimated heterogeneity in some sub-group analysis [75]. Furthermore, while analysis of SCRs can be considered a pooled analysis of individual subject data and more applicable to inference about individual responses to preEV, the analysis of the cohort GMTs is more ecological and may not be as predictive of individual responses. This may be reflected in the higher degree of heterogeneity observed for cohort GMTs compared to SCRs.

DISCUSSION

This analysis identified several trends that may support changes to the current recommendations for preEV. Previous studies have evaluated the potential of new vaccines, alternate routes, and expedited schedules to prime the immune system against rabies and elicit a long lasting immunity in all recipients. Unlike vaccines for most other infectious diseases, the primary goal of rabies preEV is to simplify rabies PEP and potentially to protect individuals from unrecognized rabies exposures (as opposed to a population prevention perspective). Due in part to this conservative approach towards rabies vaccination recommendations; there have not been major changes to the recommended schedule since the early 1980s [76]. However, findings suggestive of long lasting immunity in excess of 10 years and the high degrees of variation in the SCR observed among different studies warrant continued evaluation [32,58,77,78]. The role of routine titers in monitoring individual responses and what constitutes an 'adequate' titer also warrants additional examination. Evaluation of the current evidence for recommendations related to rabies preEV is critical to ensure that effective administration routes and schedules are recommended which meet the objectives of

rabies preEV and reduce unnecessary procedures, time, and cost to patients.

The IM route was more robust at achieving high primary SCRs regardless of the vaccine, dose, or schedule used. The lack of significant heterogeneity observed by vaccine type would appear to further confirm previous reviews which have not found a significant effect of vaccine potency on RVNA response [6]. In contrast, estimated primary SCRs for preEV by the ID route were consistently lower with higher degrees of variance between cohorts. In particular estimated SCRs for PCEC were consistently lower by the ID administration route for both the primary response and at 1-year post vaccination. Other studies have identified equivalent or higher seroconversion rates for recipients of PCECV at 2 years post-vaccination, but these studies have primarily utilized the IM route, which would be consistent with our findings [13,32]. Overall, there appears to be sufficient evidence to support current ACIP recommendations that vaccines meeting WHO potency guidelines are effectively interchangeable when required if administered by the IM route and moderate deviations in the recommended schedule are not likely to significantly impact RVNA response [5]. However, selection of vaccine type may play a more important role when the ID route is used to administer preEV.

When controlling for vaccine type, there was no significant differences in SCR between administration routes. This finding might reduce anxiety about administering vaccines by the ID route, as the equivalent SCR response observed by the SC route would suggest minimal impact if an ID dose were to be inappropriately administered SC. While Sudarshan et al. did not find significant associations between total antigenic load administered ID and RVNA response, they only examined studies that administered multi-site ID schedules which more closely approximates the antigenic dosage administered by IM routes [79]. In our study, fewer cohorts were available to

evaluate differences between single- or multi-site ID administration regimens. However, comparison of single-site ID administration using the PVRV (which provides twice the antigenic dose) at 1-year found a higher SCR compared to ID and moderately higher SCR to IM administration. Comparisons of the single- and 2-site ID administration of PCEC vaccine also identified a higher estimated SCR for the 2-site route. These findings support the bio-equivalence of ID and IM vaccination routes, which has been documented for rabies and influenza vaccines [80]. However, use of PVRV or multi-site administration routes produce a higher SCR and GMT relative to standard IM schedules, which appear to have a longer duration of immunity. Subsequently, preEV by the IM route may be considered preferable in situations where maintaining a high relative RVNA titer over a longer period is desired.

Several studies have evaluated expedited and/or reduced dose PreEV or PEP schedules [43,45,70,81-84]. A general dose response between SCRs and doses of vaccine was observed. This corresponds with early studies that found 3-doses were essential to ensure 100% seroconversion [11,76]. However, high estimated SCR were observed for shorter schedule lengths, particularly one year following vaccination (for both IM and ID administration routes). Recently published preEV studies and several studies on PEP schedules have found similar high SCR for a 3-dose one-week schedule [81-83]. Shorter vaccination schedules have the potential to facilitate better adherence to recommendations and completion of vaccination series once initiated [85]. In addition, a one week series would more closely harmonize the preEV and PEP schedules. This would make determinations of vaccination status more straightforward for persons that are recommended to discontinue PEP. Additional research would be needed to determine the impact of receiving rabies immunoglobulin on duration of immunity from

a 3-dose series. However, Naranaya et al. evaluated an ID one-week PEP schedule and did not find a significant difference in RVNA response between groups that received equine rabies immune globulin and those that did not at one-year post vaccination [81].

While adjustments to the preEV route, dose, and schedule can ensure primary seroconversion and longer duration of RVNA, it does not appear to have a significant impact on response to booster vaccination. All subjects across more than 60 cohorts developed an anamnestic response after receiving either a single booster dose or a two dose simulated PEP regimen. This review found a negative correlation between pre-booster titers and fold-change in RVNA responses 7 and 14 days post booster. Overall this resulted in cohorts achieving similar post booster GMTs regardless of the starting titer. Overall these findings provide strong support for the effectiveness of preEV and that anamnestic response to a booster appears to be robust regardless of the circumstances of the primary vaccination administration.

Nearly all studies used the WHO recommended titer of 0.5IU/mL to determine seroconversion. Currently ACIP uses a lower cut-off of approximately 0.11IU/mL, however, other values have been used in the past [5,86]. Determination of a cut-off value for managing vaccinated persons is largely dependent on the objective of conducting the serologic test. A titer of 0.5IU/mL 2-weeks after completing vaccination may not be indicative of a normal immune response. Similarly if booster response can be considered universal among immunocompetent individuals the relative value of a titer above or below 0.5IU/mL may make routine titers redundant. Based on the documented response to booster vaccination, routine titers may not be necessary on the current schedule recommended. At least one study has identified a clinical prediction model for duration of immunity based on serological monitoring at 1-year [77]. Additional evaluation

of prediction models to inform monitoring and cost effectiveness of serological modeling is warranted.

Overall, this review has provided support for several of the current recommendations and has identified evidence that shortening the current schedule and changes to serological monitoring guidelines may be possible. Additional cost-effectiveness evaluations of these potential changes should be explored to provide evidence of the impact they may have on current risk populations routinely recommended preEV. Based on this review, there does not appear to be evidence that ID and shortened preEV schedules are inferior to currently recommended schedules by ACIP and WHO, though RVNA responses may be more variable by the ID route and careful considerations should be made regarding vaccine type and schedule. Where possible multi-site ID regimens or use of PVRV is warranted if the IM route is not used.

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TABLES

Table 1. Characteristics of Selected Studies

Study (Year)	Study Design	Region	Study Population	Total Participants (n)	Study cohorts	Vaccines				Routes			Total Followup (days)	
						PCEC	PVRV	HDCV	Other	IM	ID	SC	Pre-booster	Post booster
Hafkin(1978)	Randomized Trial	USA	Veterinary School	34	2				X			X	97	n/a
Zitek(1981)	Case Series	USA	Veterinary School	66	1			X				X	369	n/a
Dreesen(1982)	Non-random trial	USA	Veterinary School	86	1			X			X		49	n/a
Burridge(1982)	Non-random trial	USA	Veterinary School	75	1			X			X		56	n/a
Barnard(1982)	Randomized Trial	USA	Veterinary School	135	5			X		X	X	X	90	n/a
Dreesen(1984)	Randomized Trial	USA	Veterinary School	60	2			X		X	X		49	n/a
Bachmann(1985)	Case Series	Switzerland	Veterinary School	44	1			X	X			X	170	n/a
Vodopija(1986)	Case Series	Yugoslavia	Community	92	4	X	X	X	X	X			90	n/a
Pappaioanou(1986)	Randomized Trial	USA	Veterinary School	25	1			X			X		105	n/a
Rodrigues(1987)	Case Series	India	At Risk	22	1			X				X	365	1825
Horman(1987)	Case Series	USA	At Risk	76	1			X			X		730	21
Fishbein(1987)	Randomized Trial	USA	Veterinary School	154	6			X		X	X		90	n/a
Morrison(1987)	Case Series	USA	At Risk	40	1			X			X		730	n/a
Bernard(1987)	Randomized Trial	USA	Veterinary School	124	5			X		X	X	X	730	21
Ajjan(1989)	Randomized Trial	France	Veterinary School	145	2		X	X		X			588	n/a
Fishbein(1989)	Randomized Trial	USA	Veterinary School	176	6			X	X	X	X		730	365
Dreesen(1989)	Randomized Trial	USA	Veterinary School	78	4	X		X		X	X		756	7
Kitala(1990)	Non-random trial	Kenya	Veterinary School	80	2		X	X		X			49	n/a
Arai(1991)	Non-random trial	Japan	Travelers	30	1	X						X	~250	~250
Hacibektasogiu(1992)	Randomized Trial	Turky	At Risk	60	2		X	X		X			60	n/a
Briggs(1996)	Non-random trial	USA	Veterinary School	146	1			X		X			360	21
Lang(1997)	Randomized Trial	Vietnam	Children	41	1		X			X			90	n/a
Lang(1998)	Randomized Trial	France	Veterinary School	330	2		X			X			365	14
Sabchareon(1998)	Randomized Trial	Thailand	Children	190	2		X			X	X		365	730
Strady(1998)	Prospective Cohort	France	At Risk	312	4		X	X		X			365	3650
Jaijaroensup(1999)	Randomized Trial	Thailand	Veterinary School	138	5	X				X	X		360	14
Lang(1999)	Prospective Cohort	Vietnam	Children	235	2		X			X	X		60	n/a
Sabchareon(1999)	Randomized Trial	Thailand	Children	400	2			X	X	X			365	14
Arora(2004)	Randomized Trial	USA	Veterinary School	135	2			X	X	X			42	n/a
Favi(2004)	Randomized Trial	Chile	Veterinary School	31	1		X			X			365	n/a
Sampath(2005)	Non-random trial	India	Community	60	1		X			X			365	n/a
Kamoltham(2007)	Randomized Trial	Thailand	Children	147	2	X					X		365	365
Khawplod(2007)	Randomized Trial	Thailand	Community	65	4		X			X	X		360	14
Shiota(2008)	Case Series	Japan	Community	20	1	X					X		208	n/a
Lalosevic(2008)	Non-random trial	Serbia	Community	120	1				X	X			37	n/a
Khawplod(2008)	Randomized Trial	Thailand	Community	52	3		X			X	X		360	14
Brown(2008)	Retrospective Cohort	UK	Community	89	1				X		X		2-10 yrs	n/a
Shanbag(2008)	Non-random trial	India	Children	175	3	X	X			X			49	n/a
Sudarshan(2008)	Randomized Trial	India	Community	20	2			X	X	X			28	n/a
Lang(2009)	Randomized Trial	Vietnam	Children	36	1		X			X			1825	n/a
Magpantay(2010)	Randomized Trial	Philippines	Community	67	1		X				X		28	n/a
Cunha(2010)	Randomized Trial	Brazil	At Risk	149	2		X			X	X		208	n/a
Sampath(2010)	Randomized Trial	India	Community	239	2		X		X	X			365	n/a
Yanagisawa(2010)	Case Series	Japan	Travelers	53	1	X						X	42	n/a
Khawplod(2012)	Prospective Cohort	Thailand	Veterinary School	109	6	X				X	X		360	14
Yanagisawa(2012)	Case Series	Japan	Community	39	1	X					X		42	n/a
Pichon(2013)	Randomized Trial	France	Community	384	2		X		X	X			365	14
Wongsaroj(2013)	Randomized Trial	Thailand	Veterinary School	55	2		X			X	X		365	14
Tantawichien(2014)	Randomized Trial	Thailand	Community	94	3		X		X	X	X		365	14
Banga(2014)	Retrospective Cohort	USA	Veterinary School	603	1	X		X		X			730	n/a
Ashwath Narayana(2014)	Non-random trial	India	Community	34	1				X	X			35	n/a

Table 2. Estimated Seroconversion Rates Following Primary Vaccination by Method of Administration*

	IM - Route					ID - Route				
	Cohorts	SCR	95% CI	I^2	p-value**	Cohorts	SCR	95% CI	I^2	p-value**
					Vaccines					
HDCV	20	99.4%	(99.0% - 99.9%)	0%	0.99	12	98.9%	(97.9% - 99.9%)	0%	1.00
PVRV	24	99.6%	(99.3% - 99.8%)	0%	0.99	6	95.4%	(91.3% - 99.4%)	92.8%	<0.00
PCECV	7	98.8%	(97.4% - 100%)	0%	0.97	13	90.5%	(85.3% - 95.6%)	85.1%	<0.00
Other	2	99.1%	(97.5% - 100%)	0%	0.67	0	-	-	-	-
					Total Vaccine Doses					
3-dose	47	99.5%	(99.3% - 99.8%)	0%	1.00	26	96.3%	(94.3% - 98.2%)	82.8%	<0.00
2-dose	5	99.5%	(98.8% - 100%)	0%	0.90	3	98.8%	(97.1% - 100%)	0%	0.85
1-dose	2	96.2%	(89.8% - 100%)	0%	0.63	2	72.7%	(58.9% - 86.5%)	0%	0.67
					Schedule Length					
28 days	37	99.5%	(99.3% - 99.8%)	0%	1.00	25	96.0%	(93.9% - 98.1%)	83.1%	<0.00
21 days	11	99.0%	(98.1% - 99.9%)	0%	1.00	3	98.3%	(95.5% - 100%)	0%	0.91
7 days	2	95.5%	(86.8% - 100%)	0%	1.00	0	-	-	-	-
	All Vaccines					PCEC Excluded				
					Administration Route					
IM	54	99.5%	(99.3% - 99.7%)	0%	1.00	46	99.5%	(99.3% - 99.8%)	0%	1.00
ID	20	97.0%	(94.9% - 99.0%)	74.3%	<0.00	12	98.9%	(97.9% - 99.9%)	0%	1.00
ID-Vero	6	95.4%	(91.3% - 99.4%)	92.8%	<0.00	6	95.4%	(91.3% - 99.4%)	92.8%	<0.00
IDx2	5	92.0%	(84.8% - 99.2%)	64.0%	0.03	0	-	-	-	-
SC	7	98.9%	(97.6% - 100%)	0.0%	1.00	6	98.8%	(97.3% - 100%)	0%	0.99

*Pooled SCRs using random effects model **Chochran's Q Test

Table 3. Estimated Seroconversion Rates One-Year Following Vaccination by Method of Administration*

	IM					ID				
	Cohorts	SCR	95% CI	I^2	p-value**	Cohorts	SCR	95% CI	I^2	p-value**
					Vaccine					
HDCV	6	55.6%	(5.6% - 100%)	99.9%	<0.00	4	48.4%	(0% - 100%)	99.5%	<0.00
PVRV	17	51.6%	(29.2% - 74.1%)	99.9%	<0.00	8	55.1%	(28.6% - 81.6%)	98.8%	<0.00
PCECV	4	38.7%	(0% - 93.3%)	98.8%	<0.00	11	17.6%	(9.8% - 25.4%)	79.5%	<0.00
					Total Vaccine Doses					
3-dose	23	54.9%	(37.7% - 72.1%)	99.9%	<0.00	18	43.9%	(24.8% - 62.9%)	98.6%	<0.00
2-dose	2	43.1%	(35.0% - 51.3%)	24.9%	0.25	2	3.9%	(0% - 9.5%)	65.7%	0.088
1-dose	2	8.0%	(0% - 17.2%)	0.0%	0.51	3	14.9%	(0.8% - 28.9%)	57.9%	0.093
					Schedule Length					
28 days	21	51.8%	(34.0% - 69.7%)	99.9%	<0.00	15	36.7%	(18.3% - 55.0%)	98.6%	<0.00
21 days	2	50.5%	(0% - 100%)	99.7%	<0.00	3	17.0%	(0% - 38.3%)	83.0%	<0.00
7 days	2	80.0%	(67.6% - 92.4%)	0.0%	1.00	2	93.70%	(85.4% - 100%)	0.0%	1.000
	All Vaccines					PCEC Excluded				
					Aministration Route					
IM	27	50.7%	(34.8% - 66.5%)	99.9%	<0.00	23	52.70%	(35.6% - 69.7%)	99.9%	<0.00
ID	11	28.4%	(5.3% - 51.4%)	98.8%	<0.00	7	48.40%	(0% - 100%)	99.5%	<0.00
ID-Vero	7	57.3%	(28.9% - 85.7%)	98.9%	<0.00	7	57.30%	(28.9% - 85.7%)	98.9%	<0.00
IDx2	5	26.0%	(10.2% - 41.8%)	76.3%	<0.00	1	-	-	-	-
SC	2	50.2%	(0% - 100%)	99.8%	<0.00	2	50.20%	(0% - 100%)	99.8%	<0.00

*Pooled SCRs using random effects model **Chochran's Q Test

Table 4. Pre- and post-booster rabies virus neutralizing antibody response

Study / Cohort	Primary series (Route / Schedule)	Pre-Booster Titer Day	N	Pre-Booster GMT	Pre-Booster SCR	Booster (route/schedule)	Post-booster GMT (day 5-7)	Post-booster GMT (day 14-20)	GMT Fold Increase (7 days)	GMT Fold Increase (14 days)
Hafkin(1978) / 3	SQ / 0	84	15	0.17	<93	SQ / 0	-	1.64	-	9.6
Hafkin(1978) / 2	SQ / 0,7,14	84	19	2.7	100	SQ / 0	-	15.17	-	5.6
Bachmann(1985) / 1	SQ / 0,28	120	44	2.7	95.5	SQ / 0	-	7.9	-	2.9
Kamoltham(2007) / A	ID / 0,28	365	84	0.11	7	ID / 0,3	4.69	10.76	42.6	97.8
Khawplod(2012) / 1a	ID / 0,7,21	360	17	0.49	29	IM / 0,3	11.27	54.53	23.0	111.3
Khawplod(2012) / 1b	ID / 0,7,21	360	19	0.3	26	IDx4 / 0	42.49	114.28	141.6	380.9
Jaijaroensup(1999) / B	ID / 0,7,28	365	21	0.11	6	ID / 0,3	0.27	5.03	2.5	45.7
Jaijaroensup(1999) / C	ID / 0,7,28	365	22	0.15	14	IDx2 / 0,3	0.54	19.71	3.6	131.4
Kamoltham(2007) / B	ID / 0,7,28	365	63	0.33	35	ID / 0,3	10.96	22.12	33.2	67.0
Bernard(1987) / 2	ID / 0,7,28	365	11	1.5	92	ID / 0	-	42.5	-	28.3
Bernard(1987) / 3	ID / 0,7,28	365	10	0.6	<90	ID / 0	-	32.8	-	54.7
Khawplod(2012) / 2a	IDx2 / 0	360	16	0.15	5	IM / 0,3	9.71	46.23	64.7	308.2
Khawplod(2012) / 2b	IDx2 / 0	360	24	0.1	13	IDx4 / 0	11.96	54.36	119.6	543.6
Jaijaroensup(1999) / D	IDx2 / 0,7,28	365	21	0.46	43	ID / 0,3	0.68	16.38	1.5	35.6
Jaijaroensup(1999) / E	IDx2 / 0,7,28	365	25	0.42	72	IDx2 / 0,3	1.07	29.84	2.5	71.0
Khawplod(2007) / D	IDx2V / 0	360	13	0.41	38	ID / 0,3	9.15	51.96	22.3	126.7
Khawplod(2008) / B	IDx2V / 0,3,7	360	16	1.12	94	IDx2 / 0,3	22.99	105.1	20.5	93.8
Khawplod(2007) / B	IDx2V / 0,3,7	360	16	1.12	94	ID / 0,3	22.99	105.1	20.5	93.8
Khawplod(2008) / A	IDx2V / 0,7,28	360	16	0.96	81	IDx2 / 0,3	29.14	49.4	30.4	51.5
Khawplod(2007) / A	IDx2V / 0,7,28	360	16	0.96	81	ID / 0,3	29.14	49.4	30.4	51.5
Wongsaroj(2013) / A	IDxV / 0,21	365	36	0.35	<97	ID / 0,3	-	14.28	-	40.8
Tantawichien(2014) / A	IDxV / 0,7,28	365	32	0.28	<93	ID / 0,3	-	11.93	-	42.6
Sabchareon(1998) / 1	IDxV / 0,7,28	365	80	0.4	52.5	ID / 0	11.8	-	29.5	-
Khawplod(2012) / 3a	IM / 0	360	17	0.08	6	IM / 0,3	10.13	18.96	126.6	237.0
Khawplod(2012) / 3b	IM / 0	360	16	0.11	13	IDx4 / 0	13.33	46.92	121.2	426.5
Strady(1998) / 1	IM / 0,28	365	78	1.2	38.5	IM / 0	-	46	-	38.3
Strady(1998) / 2	IM / 0,28	365	111	1.3	46.6	IM / 0	-	24	-	18.5
Khawplod(2008) / C	IM / 0,3,7	360	20	0.97	80	IDx2 / 0,3	35.16	125	36.2	128.9
Khawplod(2007) / C	IM / 0,3,7	360	20	0.97	80	ID / 0,3	35.16	125	36.2	128.9
Wongsaroj(2013) / B	IM / 0,7,21	365	15	0.76	<93	ID / 0,3	-	14.06	-	18.5
Briggs(1996) / 1	IM / 0,7,28	360	146	7.3	<99	IM / 0	12.84	-	1.8	-
Jaijaroensup(1999) / A	IM / 0,7,28	365	21	0.45	38	IM / 0,3	1.47	19.89	3.3	44.2
Lang(1998) / 1	IM / 0,7,28	365	158	3.5	97.5	IM / 0	-	26.2	-	7.5
Lang(1998) / 2	IM / 0,7,28	365	163	5.2	99	IM / 0	-	42.4	-	8.2
Tantawichien(2014) / B	IM / 0,7,28	365	31	0.64	<96	IM / 0,3	-	45.99	-	71.9
Tantawichien(2014) / C	IM / 0,7,28	365	31	0.55	<96	IM / 0,3	-	29.16	-	53.0
Pichon(2013) / A*	IM / 0,7,28	365	230	0.67	<99	IM / 0	-	27.1	-	40.4
Pichon(2013) / B	IM / 0,7,28	365	118	0.97	<99	IM / 0	-	28.4	-	29.3
Sabchareon(1999) / 1	IM / 0,7,28	365	150	2.2	88	IM / 0	27.6	31.9	12.5	14.5
Sabchareon(1999) / 2	IM / 0,7,28	365	153	3.6	97	IM / 0	42.3	46.8	11.8	13.0
Sabchareon(1998) / 2	IM / 0,7,28	365	75	1.3	90.5	IM / 0	22.9	-	17.6	-
Strady(1998) / 3	IM / 0,7,28	365	30	2.9	100	IM / 0	-	54.1	-	18.7
Strady(1998) / 4	IM / 0,7,28	365	67	1.9	88	IM / 0	-	50.6	-	26.6
Bernard(1987) / 1	IM / 0,7,28	365	8	3.3	100	ID / 0	-	60.3	-	18.3
Bernard(1987) / 4	SQ / 0,7,28	365	10	0.6	<90	ID / 0	-	48.4	-	80.7
Bernard(1987) / 5	SQ / 0,7,28	365	9	1.6	100	ID / 0	-	26.7	-	16.7
Fishbein(1989) / 5	ID / 0,7,28	730	40	1.53	<97	ID / 0	8.81	-	5.8	-
Fishbein(1989) / 6	ID / 0,7,28	730	37	1.25	<97	ID / 0	6	-	4.8	-
Bernard(1987) / 2	ID / 0,7,28	731	11	1.7	91	ID / 0	-	7.7	-	4.5
Bernard(1987) / 3	ID / 0,7,28	731	10	0.4	<90	ID / 0	-	10.8	-	27.0
Dreesen(1989) / 3	ID / 0,7,28	756	17	0.56	<94	IM / 0	11.06	-	19.8	-
Dreesen(1989) / 4	ID / 0,7,28	756	18	0.6	<94	IM / 0	17.91	-	29.9	-
Bernard(1987) / 1	IM / 0,7,28	731	9	1.3	78	ID / 0	-	5.1	-	3.9
Dreesen(1989) / 1	IM / 0,7,28	756	17	1.68	100	ID / 0	18.69	-	11.1	-
Dreesen(1989) / 2	IM / 0,7,28	756	18	0.92	<94	IM / 0	16.37	-	17.8	-
Bernard(1987) / 4	SQ / 0,7,28	731	13	0.4	54	ID / 0	-	10.5	-	26.3
Bernard(1987) / 5	SQ / 0,7,28	731	12	0.7	<91	ID / 0	-	8.4	-	12.0
Strady(1998) / 1	IM / 0,28	4015	39	3.7	87.1	IM / 0	-	34	-	9.2
Strady(1998) / 2	IM / 0,28	4015	62	2.9	65	IM / 0	-	21.4	-	7.4
Strady(1998) / 3	IM / 0,7,28	4015	17	12.9	96.2	IM / 0	-	53.6	-	4.2
Strady(1998) / 4	IM / 0,7,28	4015	49	7.7	97	IM / 0	-	37.5	-	4.9

Figures

Figure 1. Flowchart of Study Selection Process

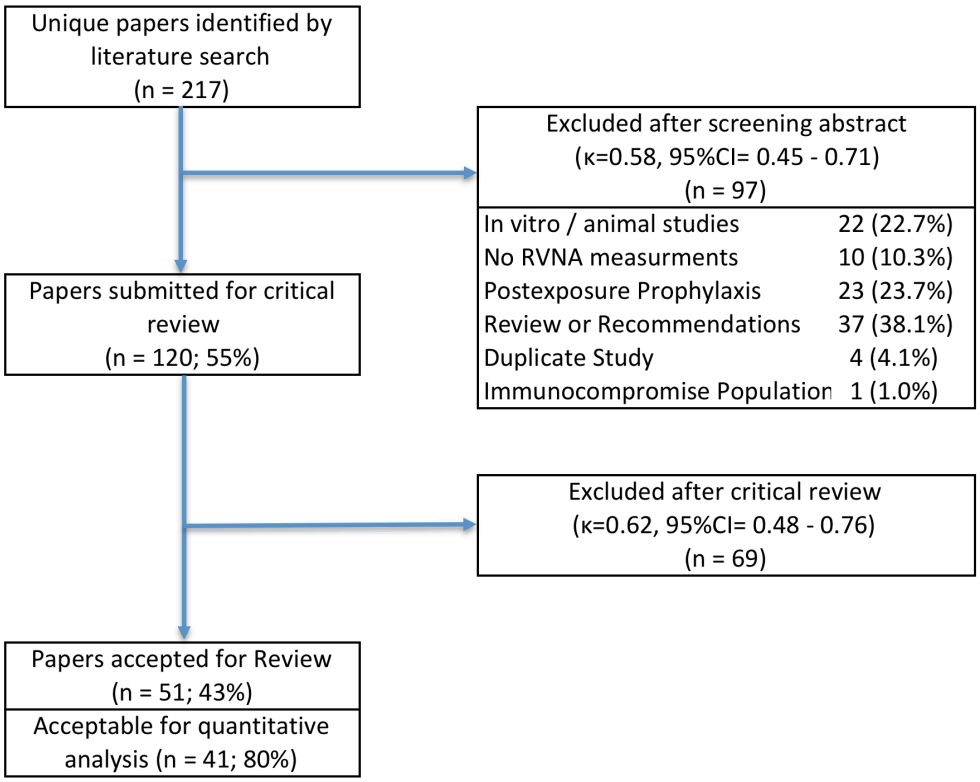


Figure 2. Forrest plots showing log transformed geometric mean titers of cohorts administered vaccine by the IM route (28, 45, and 365 days after starting vaccination).

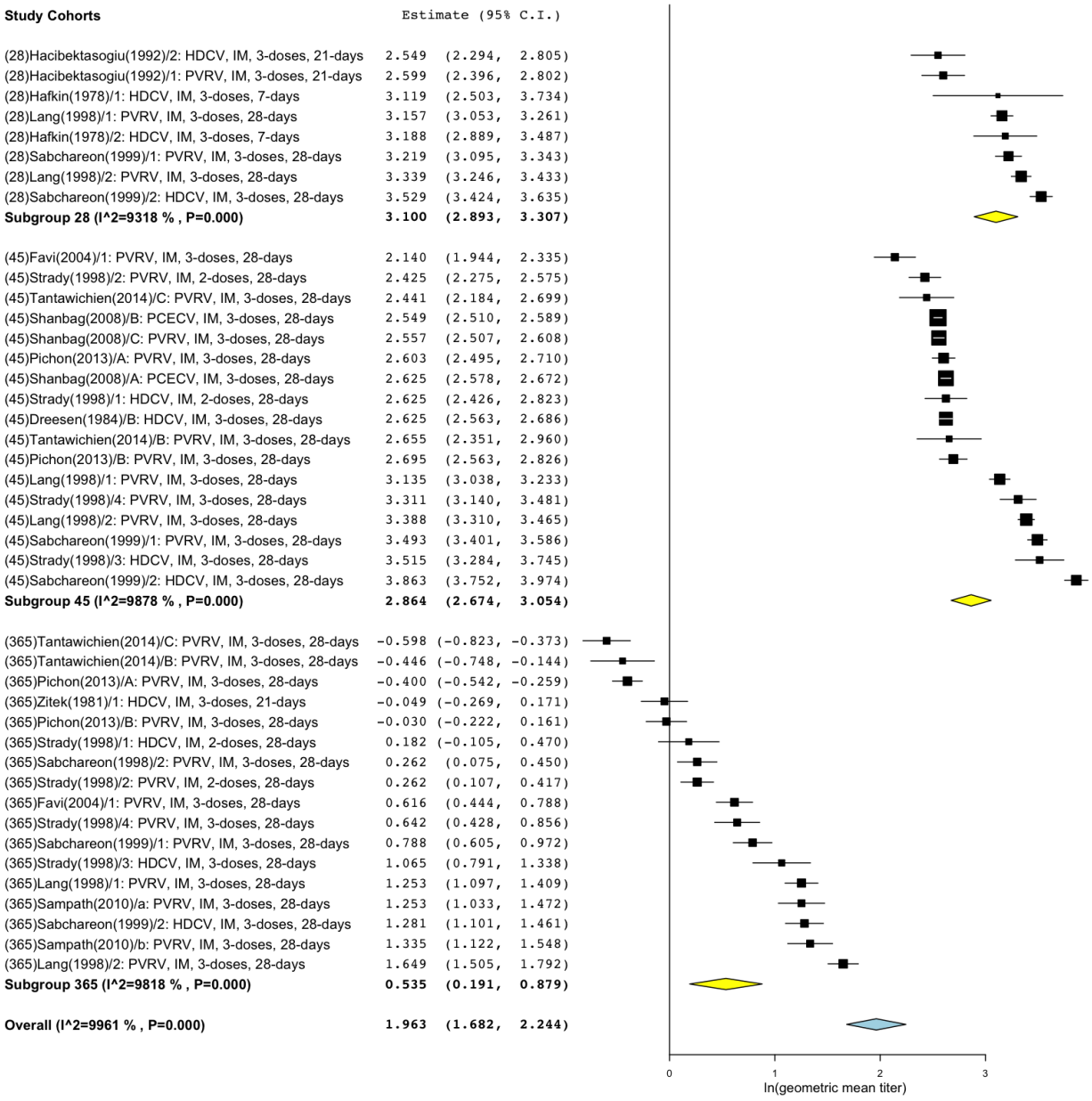


Figure 3. Summary Survival Curves of Seroconversion following Rabies Vaccination Administered by Intramuscular or Intradermal Route.

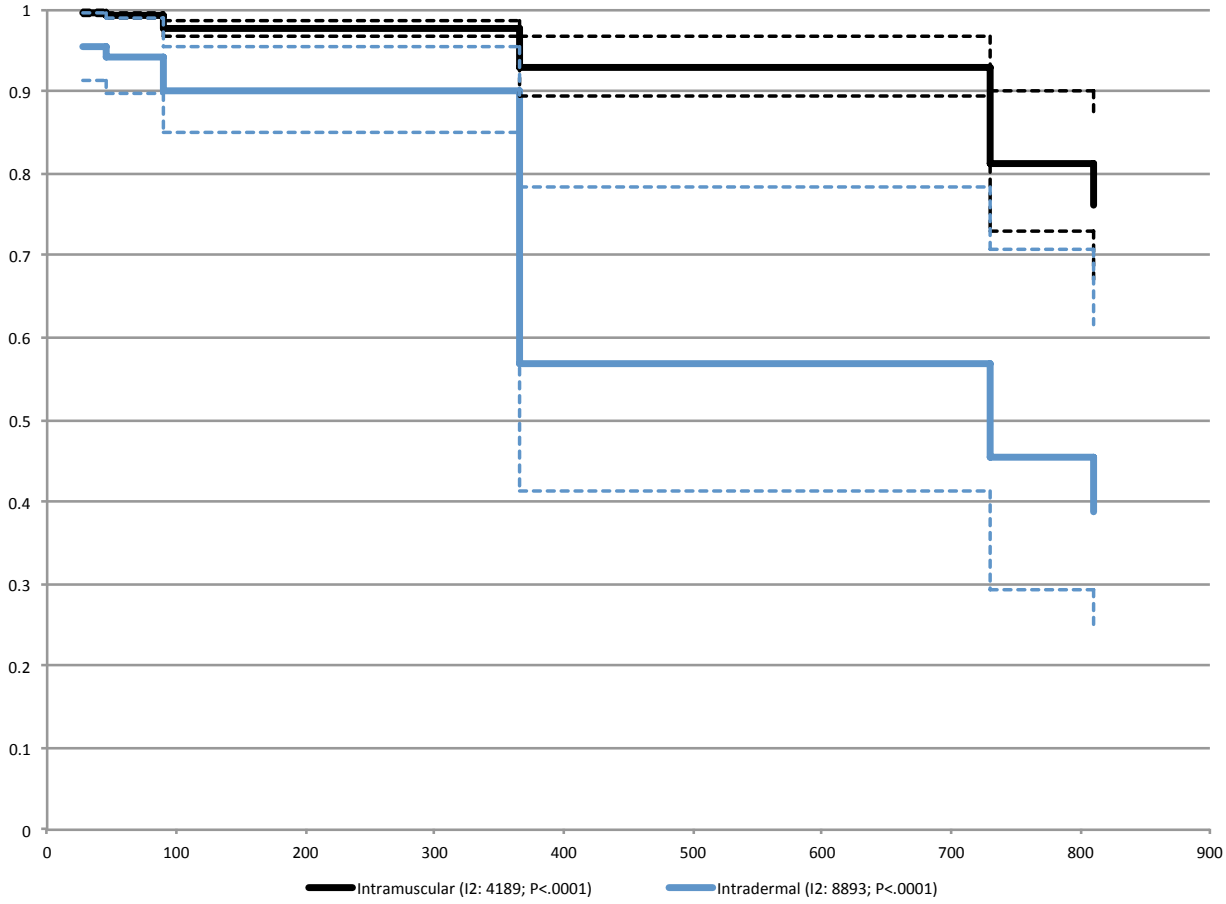
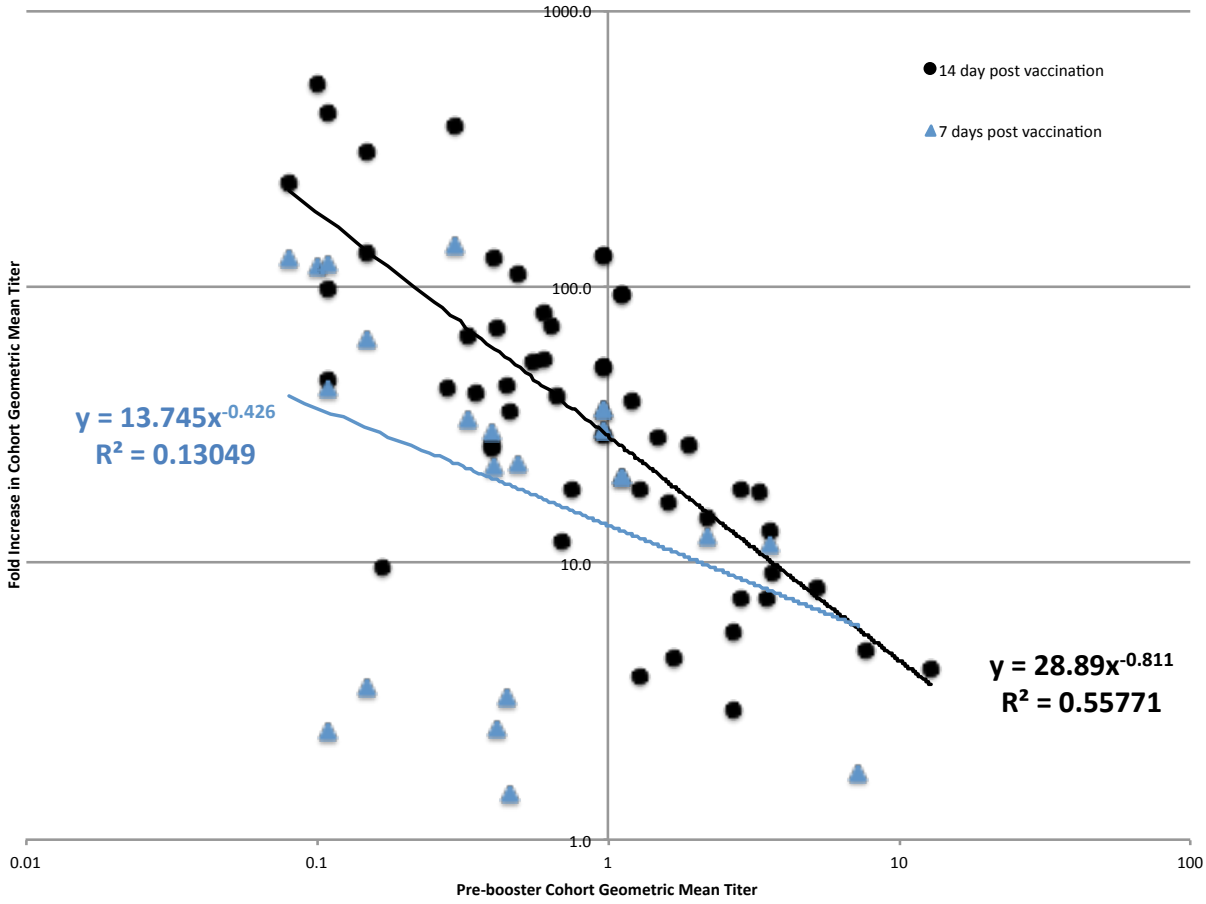
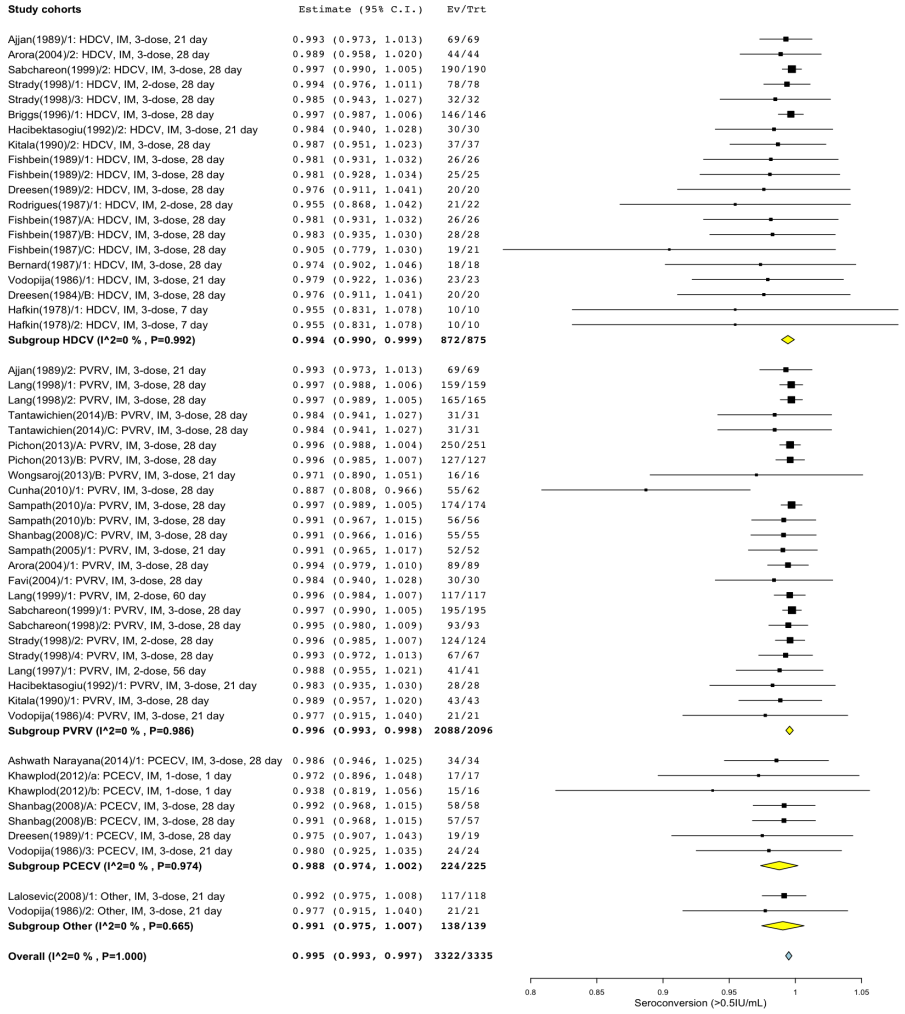


Figure 4. Fold-increase in post-booster geometric mean titer by pre-booster titer.

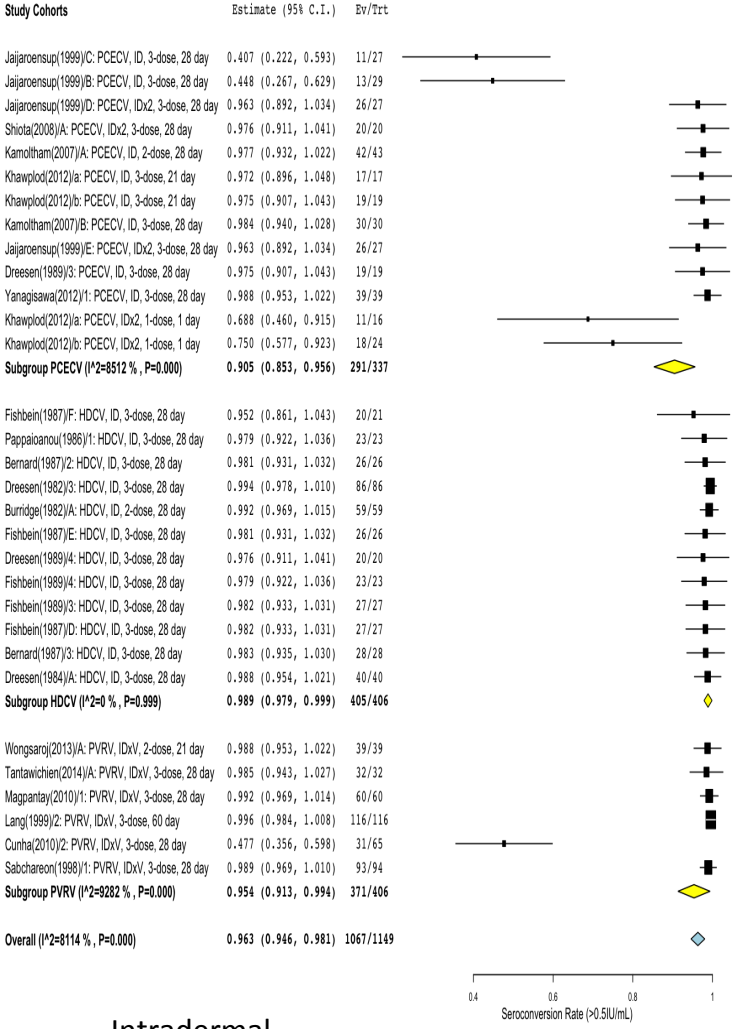


SUPPLEMENTAL FIGURES

SF1. Forest plots showing primary seroconversion rates from cohorts administered different rabies vaccines by Intramuscular and Intradermal routes.

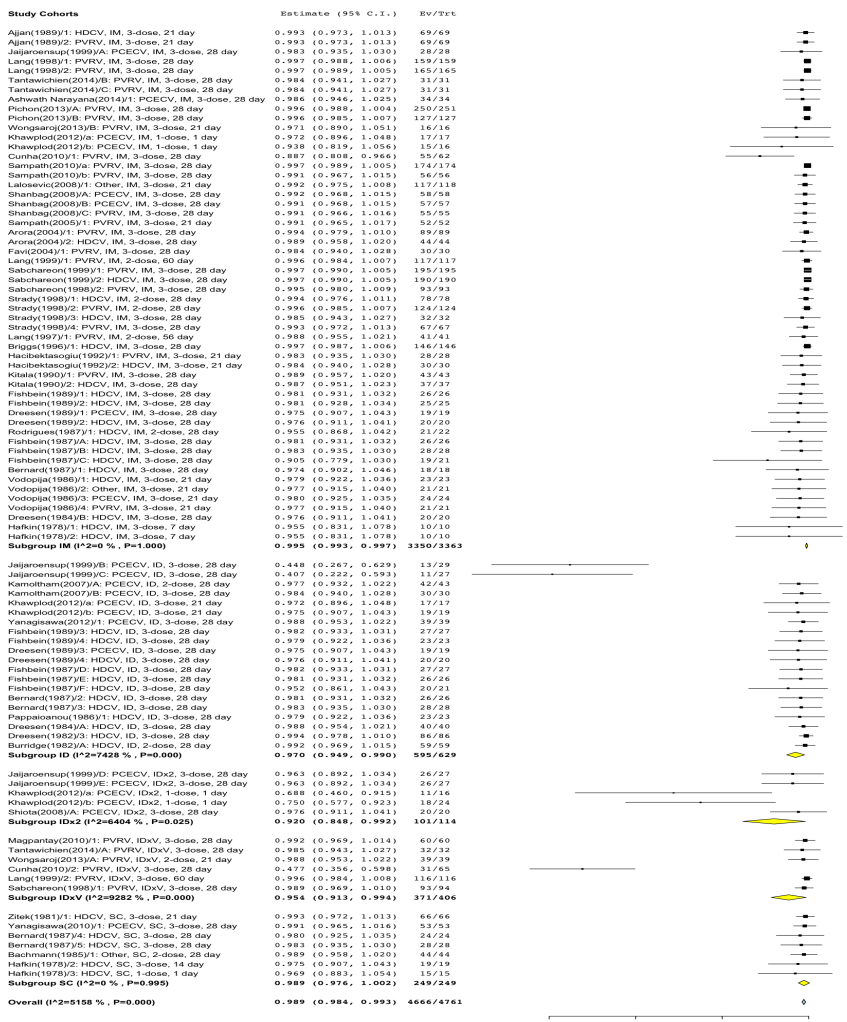


Intramuscular

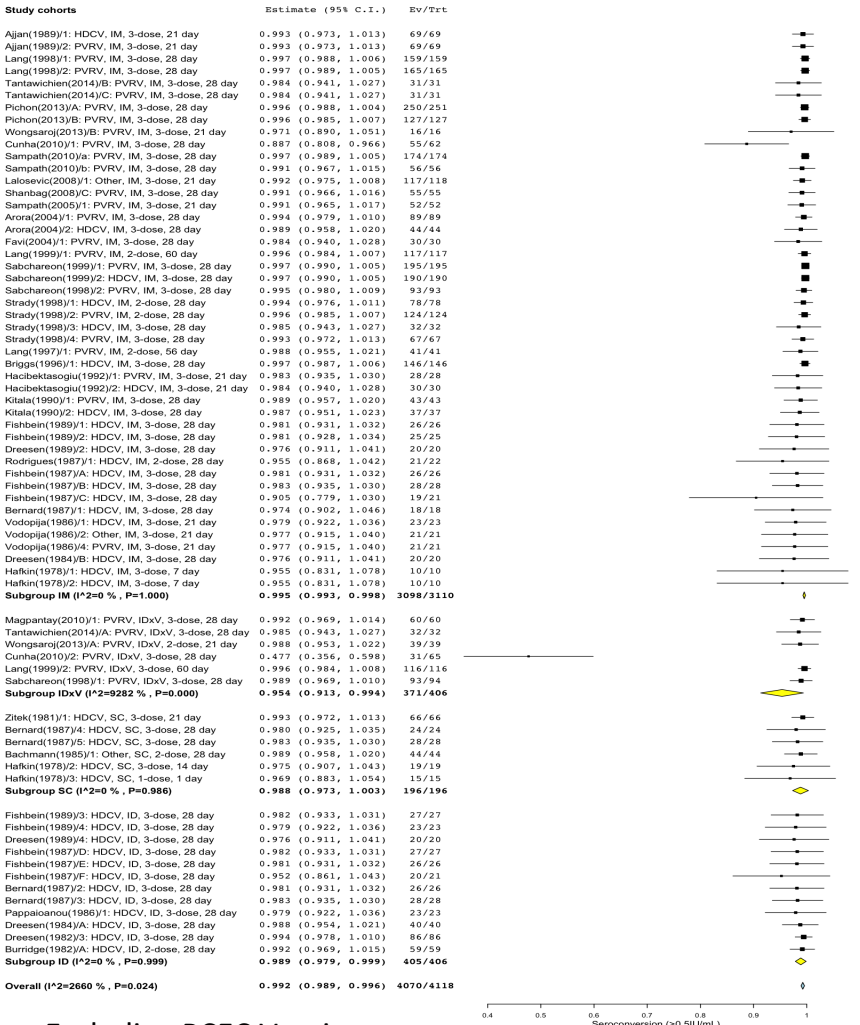


Intradermal

SF2. Forest plots showing primary seroconversion rates from cohorts administered rabies vaccine by different routes.

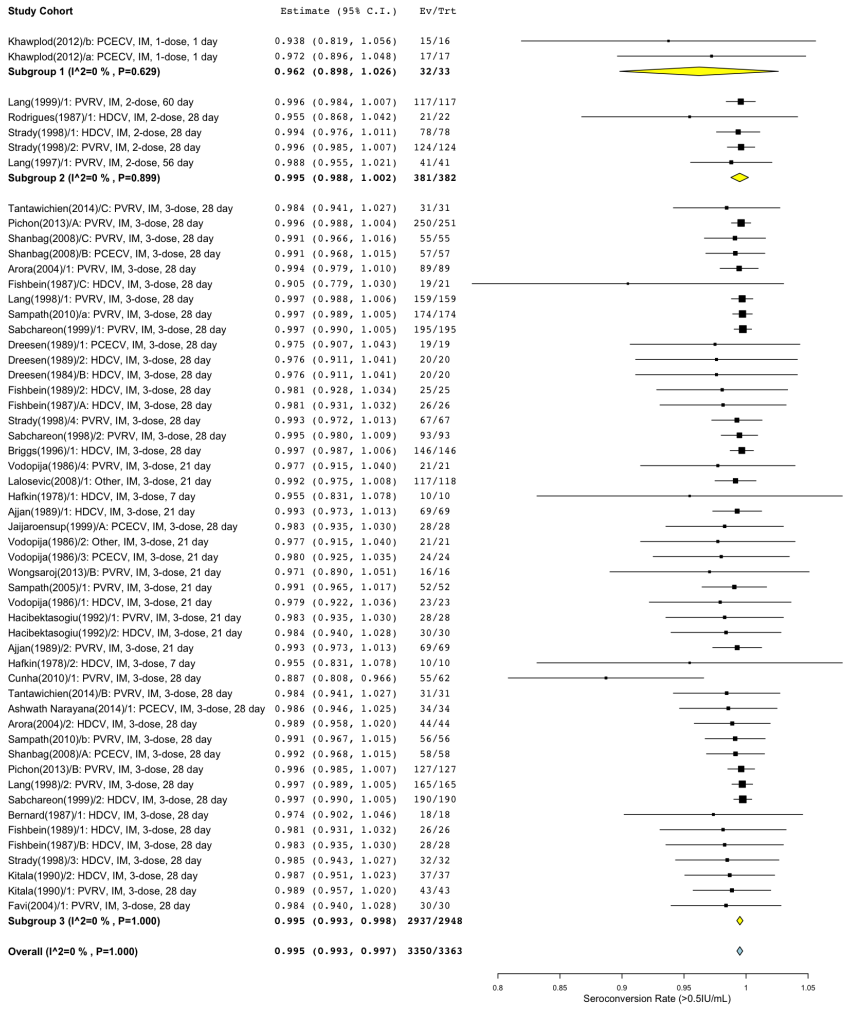


All Vaccines

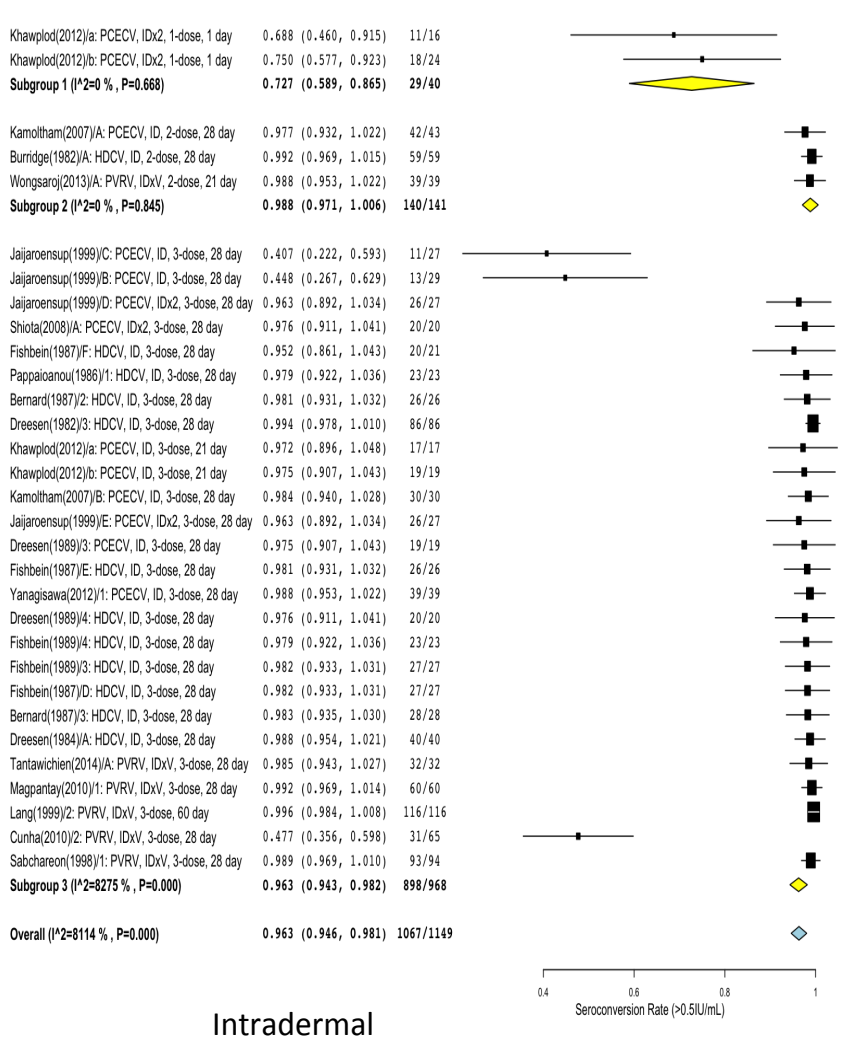


Excluding PCEC Vaccine

SF3. Forest plots showing primary seroconversion rates from cohorts administered different number of vaccine doses by Intramuscular and Intradermal routes.

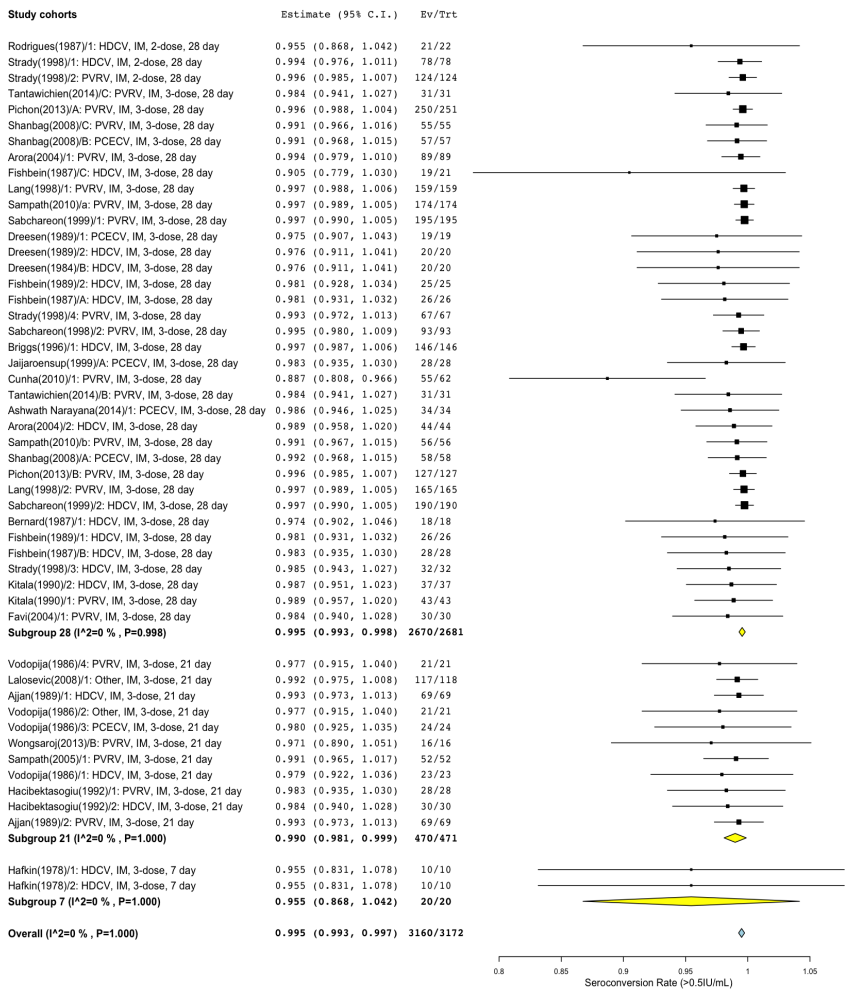


Intramuscular

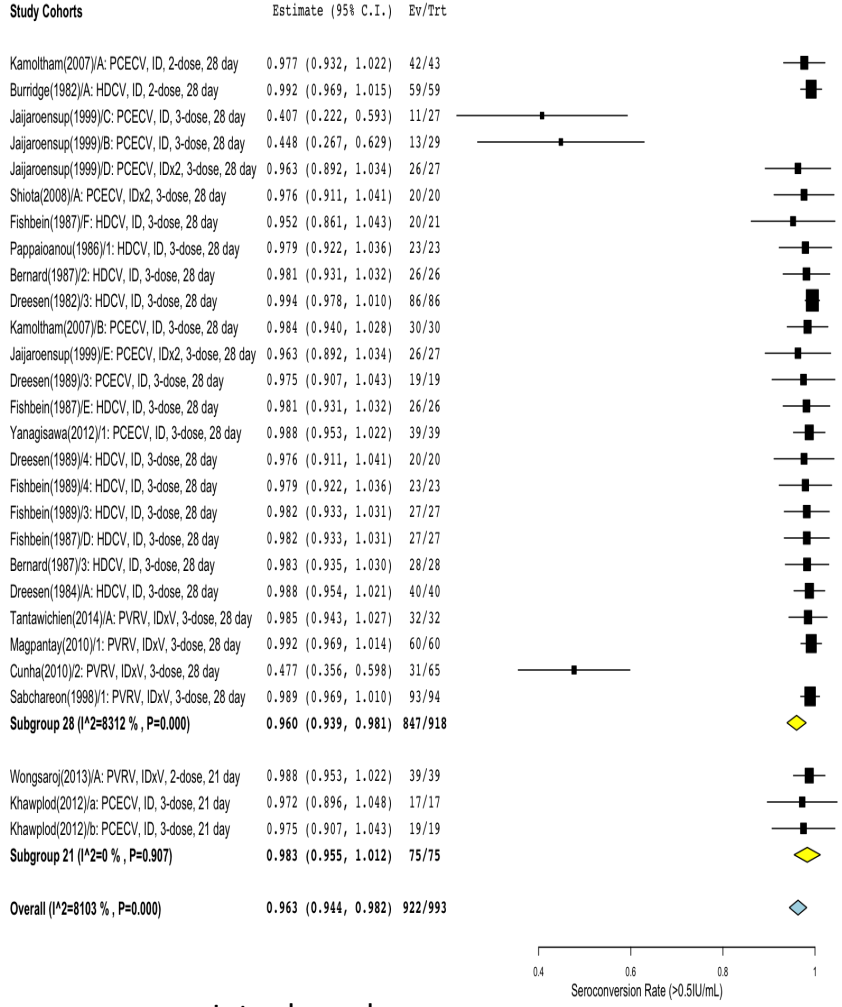


Intradermal

SF4. Forest plots showing primary seroconversion rates from cohorts administered vaccines over 7, 21, or 28 day schedules by Intramuscular and Intradermal routes.

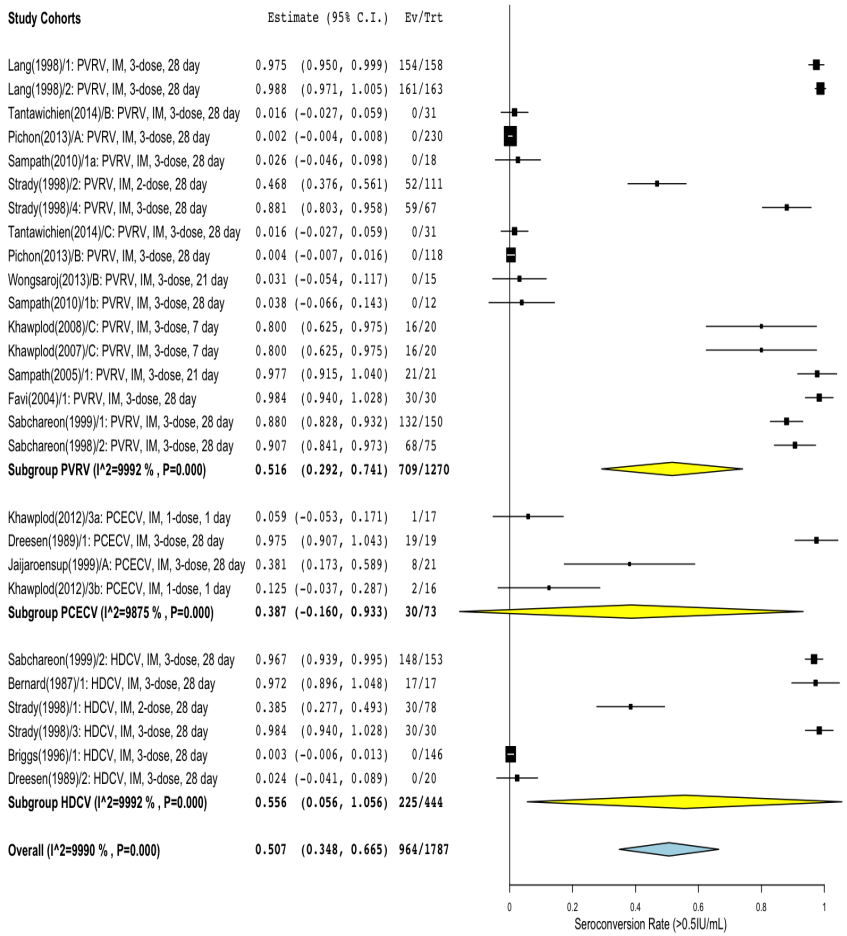


Intramuscular

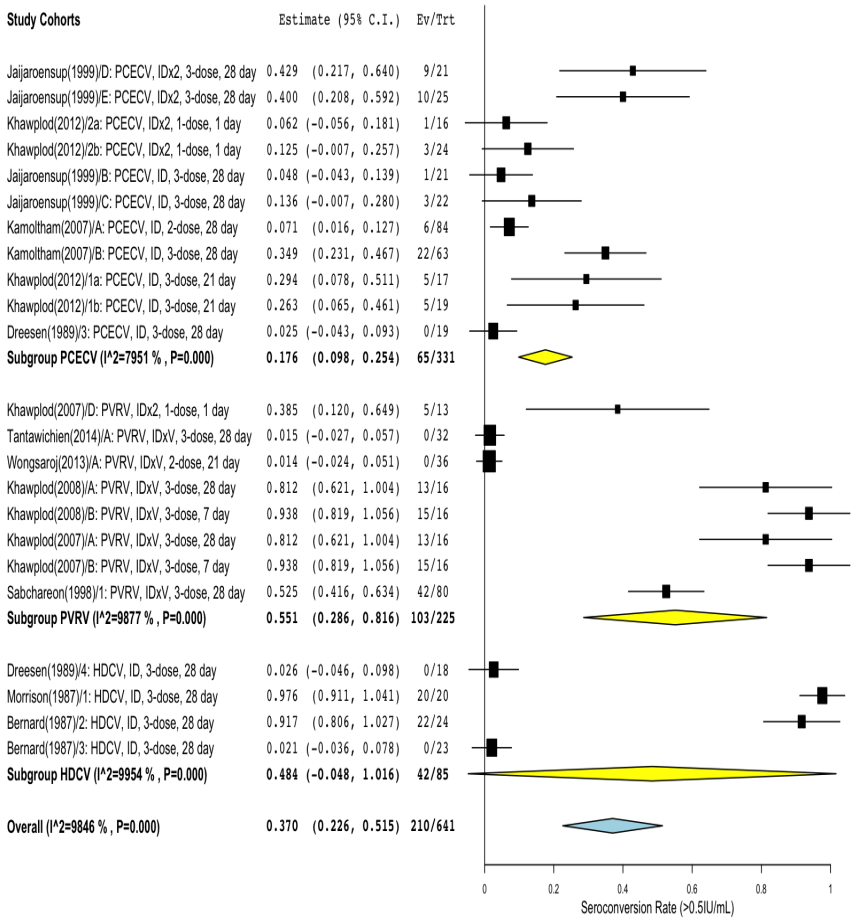


Intradermal

SF5. Forest plots showing 1-year seroconversion rates from cohorts administered different rabies vaccines by Intramuscular and Intradermal routes.

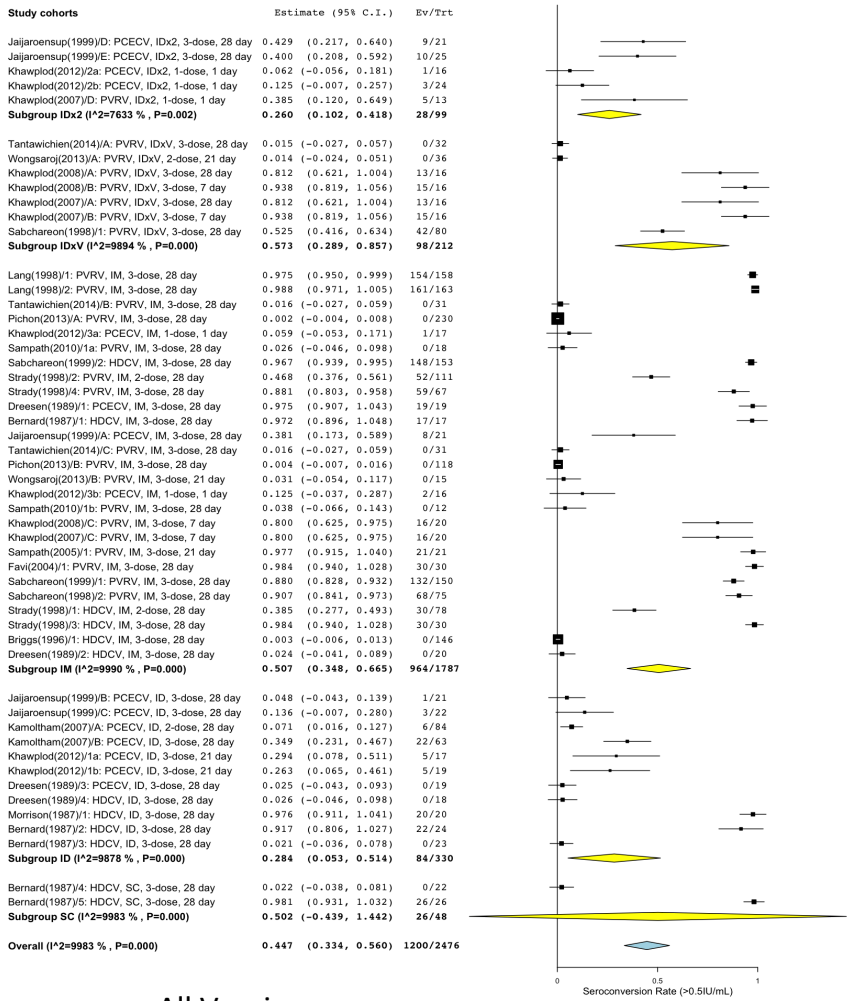


Intramuscular

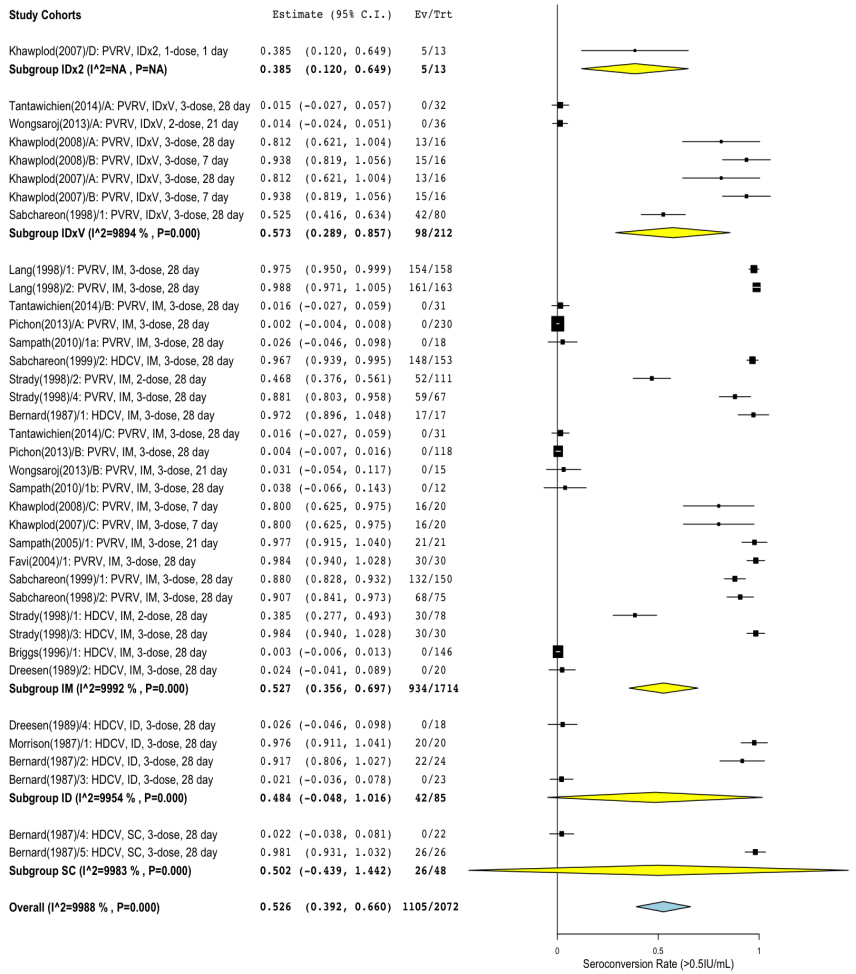


Intradermal

SF6. Forest plots showing 1-year seroconversion rates from cohorts administered rabies vaccine by different routes.

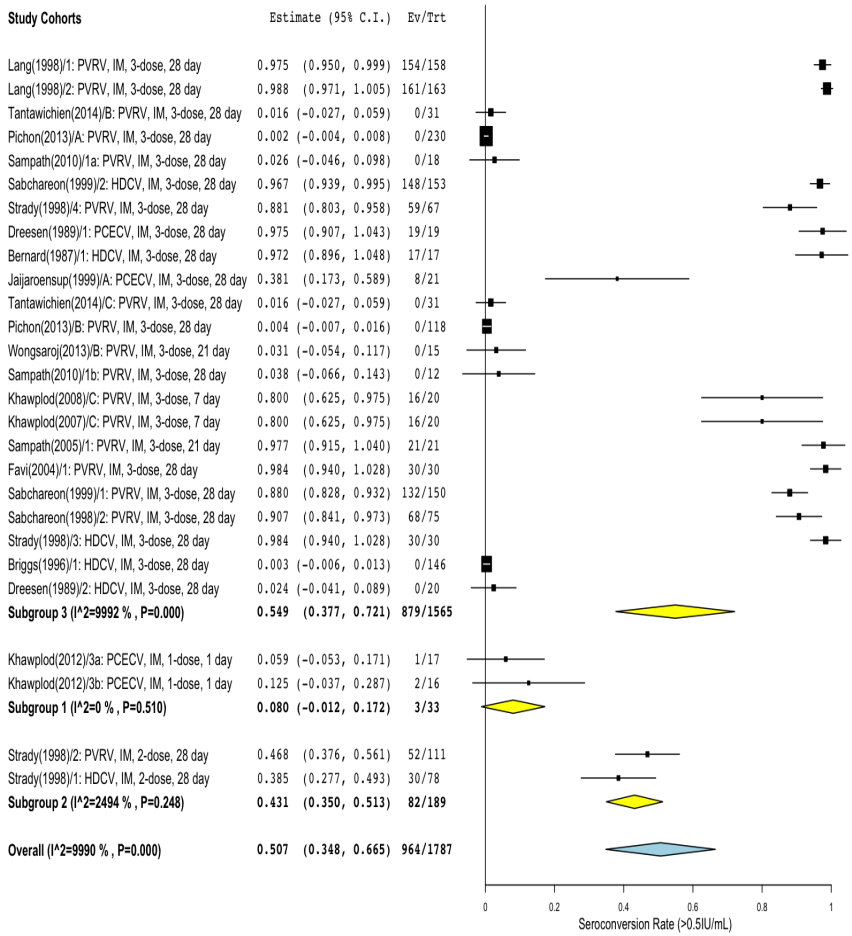


All Vaccines

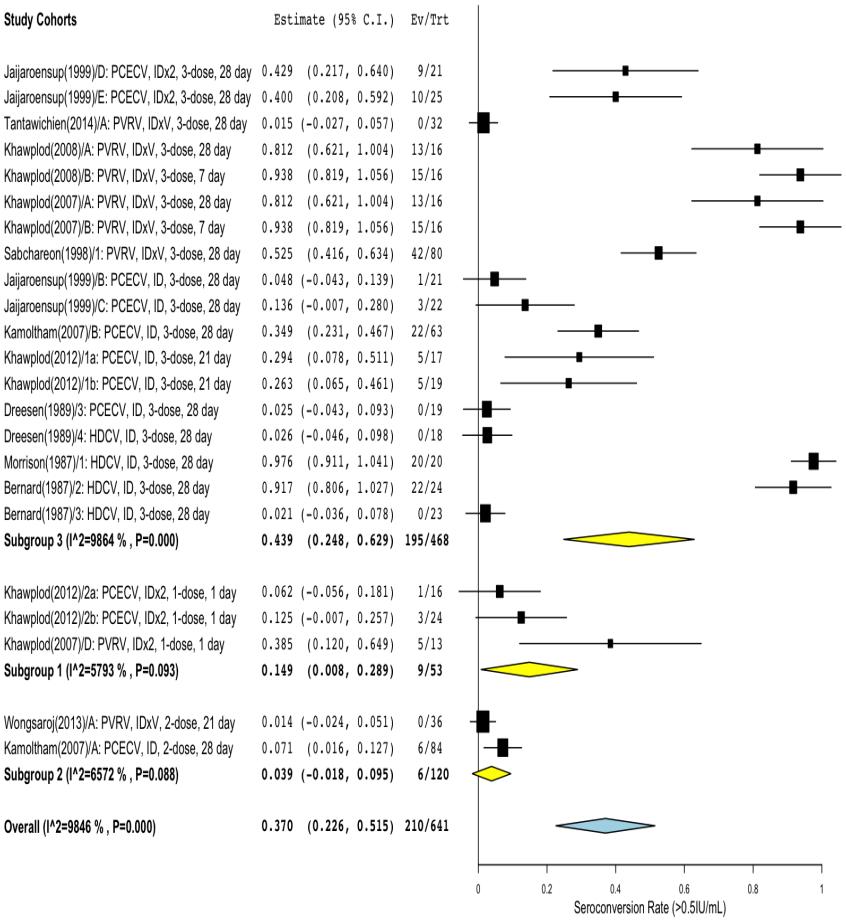


Excluding PCEC Vaccine

SF 7. Forest plots showing 1-year seroconversion rates from cohorts administered different number of vaccine doses by Intramuscular and Intradermal routes.

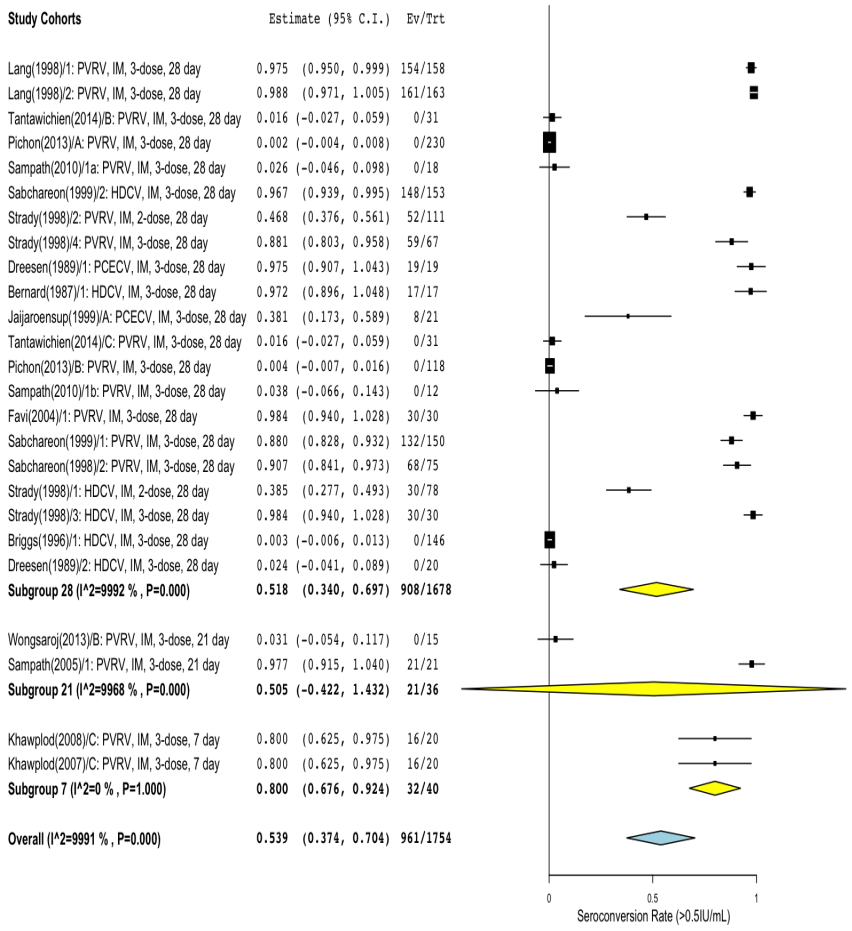


Intramuscular

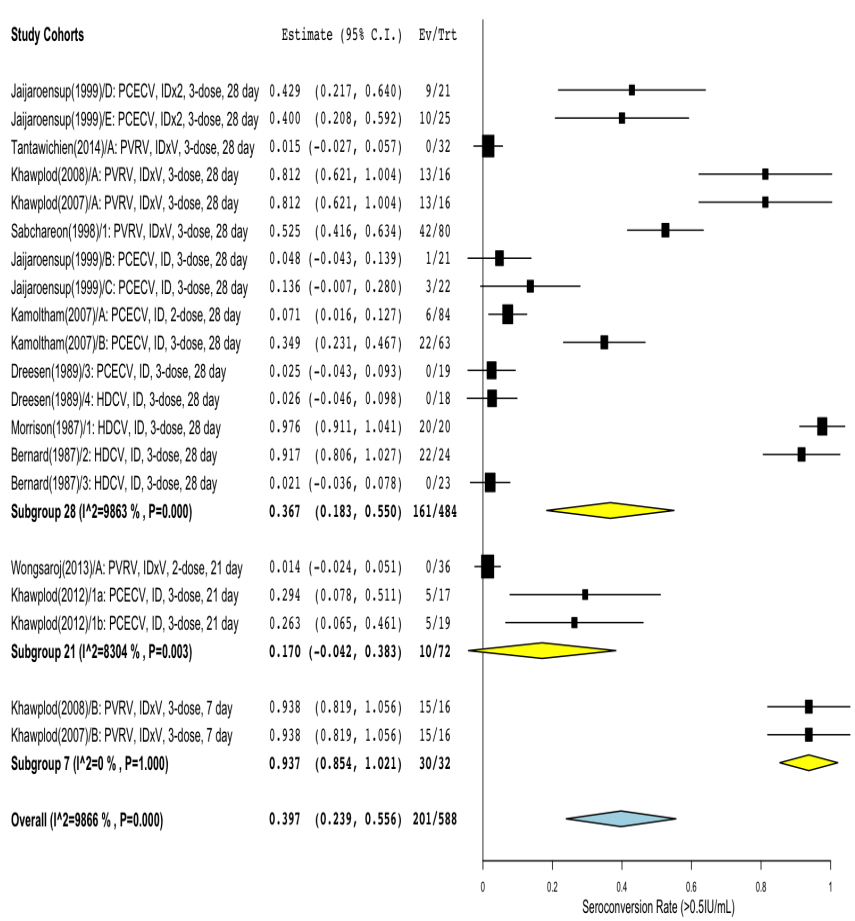


Intradermal

SF 8. Forest plots showing 1-year seroconversion rates from cohorts administered vaccines over 7, 21, or 28 day schedules by Intramuscular and Intradermal routes.



Intramuscular



Intradermal

CHAPTER 5: Conclusions

SUMMARY

The objective of this dissertation is to evaluate the current risk of rabies exposure among high-risk populations, determine the adherence to current rabies vaccination recommendations, and to review published literature for evidence regarding multiple rabies pre-exposure vaccination (preEV) schedules. Specifically, the aim was to generate evidence that will facilitate the updating of the Advisory Committee on Immunization Practices (ACIP) recommendations on human rabies prevention, which were last reviewed in 2008.

Two research projects were executed to achieve these objectives. The first consisted of a cross-sectional web-based survey distributed to populations with a high-risk of rabies exposure through their occupation. These groups were recruited through professional organizations with memberships consisting of animal control officers, veterinarians, veterinary technicians, and wildlife rehabilitators. This study was designed to estimate the rate of animal bites, rabies exposures, rabies vaccination, and serological monitoring among these different categories of animal health providers. In addition, the survey elicited information on potential factors that might be associated with adhering to current rabies vaccination and serological monitoring recommendations. The study found bite rates among animal health providers was more than 70 times higher than the estimated rate in the general population, with rabies postexposure prophylaxis (PEP) rates that were more than 100 times higher. However, rabies vaccination rates were not uniform across the groups. With the exception of veterinarians, reported vaccination rates were less than 80% in animal control officers, veterinary technicians, and wildlife rehabilitators. Similarly, only 60% of all respondents were up-to-date on serological monitoring as outlined in ACIP. Awareness of an employee policy requiring vaccination or routine serological monitoring had the largest effect on adherence to recommendations.

The second study was designed to evaluate the published literature for immune response following primary rabies preEV. A systematic review and meta-analysis was conducted to compare longitudinal seroconversion rates (SCR) and geometric mean titers (GMT) following vaccination using multiple vaccines, administration routes, and schedule lengths. The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A total of 120 studies were selected for review, of which 51 (42.5%) met inclusion criteria consisting of 112 cohorts. Overall the review found that administration of rabies vaccine by the intramuscular route (IM) was robust, producing high SCRs and GMTs, regardless of the vaccine or schedule used. Alternatively, SCRs were lower for equivalent vaccines and routes administered by the intradermal (ID) route. There was also significant heterogeneity between cohorts for most sub-group analysis by the ID route. Determination of summary survival curves based on seroconversion found a longer duration of immunity by the IM route (>810 days) compared to the ID route (584 days). However, all subjects in 61 cohorts that received a booster vaccination developed an anamnestic response regardless of the preEV series received, time since primary vaccination, or titer at time of booster. Overall, the literature review supported current ACIP and WHO recommendations, while also providing evidence to support the consideration of a shorter 1-week vaccination schedule compared to the current 3-4 week schedule. Current recommendations to conduct serological monitoring every two years in high-risk populations (e.g. veterinarians in rabies endemic areas) should be re-evaluated in context of exposure risk given the consistent serological response to booster vaccination.

LIMITATIONS

The use of professional organizations to recruit survey participants may have resulted in systematic bias and restricted generalizability to animal health provider populations in these or similar organizations. However, members of such organizations may be more engaged in their profession and receive more frequent member education opportunities and training activities. Therefore, estimates of rabies exposure and vaccination rates may be conservative. However, additional research in broader animal health provider

populations is warranted. The overall response rates were relatively low, but were consistent across organizations. Demographic information from the American Veterinary Medical Association and National Association of Veterinary Technicians in America was available to compare to respondents from each group. Distributions for sex and level of education were similar in both cases. Respondents were slightly younger in comparison to their overall organization membership, which may be a function of recruitment via email listserv and use of a web-based survey tool.

The systematic review identified a sufficient number of studies and cohorts for a robust meta-analysis. However, several sub-group analyses consisted of only 2 to 4 cohorts. This may have resulted in an underestimate of heterogeneity. Furthermore because the Rapid Fluorescent Focus Inhibition Test (RFFIT) used to determine individual titers is a cell culture based assay, inter-laboratory variation is expected. This may have introduced additional non-systematic bias between studies, potentially accounting for some of the heterogeneity reported. This is consistent with the finding of increased heterogeneity in the analysis of GMTs, compared to SCRs.

CONCLUSIONS

This research quantified rabies exposure risk to animal health workers and factors associated with their adherence to current rabies vaccination recommendations. Improvements in knowledge and awareness of rabies exposure mechanisms and vaccination recommendations are needed across all occupational groups. Recognition of these risks and gaps in knowledge are critical for the development of updated ACIP and WHO recommendations. This information should help guide targeted outreach to improve awareness of recommendations in these populations. These results suggest outreach to universities, veterinary schools, training programs, and employers to develop appropriate policies reflective of recommendations for vaccination and serological monitoring would have the greatest impact towards increasing adherence.

In addition to the findings on risk and vaccination recommendations, this research outlined evidence in the existing literature that might support changes to ACIP recommendations. Ultimately any changes to current recommendations should simplify

vaccination and monitoring processes and increase adherence while maintaining current levels of efficacy. Reducing the preEV schedule to a 3-dose 1-week series (compared to the current 21 or 28 days) does not appear to significantly reduce SCRs. Such an expedited schedule would allow persons to complete preEV quicker allowing them to start their occupational activities that require vaccination earlier. It may also increase access for travelers, who frequently are unable to start preEV due to insufficient time to complete the rabies vaccination series prior to travel. Furthermore, response to booster vaccination was universal regardless of vaccination regimen, time since vaccination, or titer at time of booster. This challenges the current recommendations for serological monitoring for certain risk groups every two years. Furthermore, approximately 40% of these groups are not currently adhering to this routine monitoring. This recommendation should be re-evaluated to clarify the objectives of monitoring this population at the current frequency stipulated.

RECOMMENDATIONS AND FUTURE RESEARCH

These results provide evidence that will aid in the development of new recommendations related to rabies vaccination. In particular these findings can provide evidence towards updating the current ACIP human rabies prevention recommendations regarding high-risk populations, serological monitoring guidelines, and stronger language to encourage vaccination policies at training institutes or workplaces. In addition, modification of the current preEV schedule to reduce the schedule length from 3 to 4 weeks to 1-week should be considered.

Future research is planned to evaluate the effects of healthcare access, costs, and willingness to pay on adherence to current ACIP recommendations. The costs of rabies vaccination and for rabies virus neutralizing antibody testing are relatively high and are anticipated to play a role in adherence to recommendations. Particularly when considering trade-offs between serological monitoring versus just receiving a periodic booster vaccination. Additional prospective studies related to the serologic response and duration of immunity following the expedited 1-week series is needed.

APPENDIX

Phone 706-542-3199

Office of the Vice President for Research
Institutional Review Board

APPROVAL OF PROTOCOL

April 28, 2016

Dear Joel Lee:

On 4/28/2016, the IRB reviewed the following submission:

Type of Review:	Initial Study
Title of Study:	Attitudes and practices towards rabies vaccination recommendations among persons with occupational risks of rabies exposure
Investigator:	Joel Lee
IRB ID:	STUDY00003431
Funding:	None
Grant ID:	None

The IRB approved the protocol from 4/28/2016.

In conducting this study, you are required to follow the requirements listed in the Investigator Manual (HRP-103).

Sincerely,

Dr. Gerald E. Crites, MD, MEd
University of Georgia
Institutional Review Board Chairperson