ANIMAL RABIES IN NEPAL AND RACCON RABIES IN ALBANY COUNTY, NEW YORK

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Abstract

Rabies is a fatal viral disease that has existed since the antiquity, and is prevalent throughout the world. Wild animals contribute to the spread of this disease to humans and animals in developed nations; however, canines are responsible in transmitting to humans, mostly in Asia and Africa. About 96% of human rabies cases are attributed to dog bites. Annually, 55,000 people (56% in Asia, 44% in Africa) in the world die of rabies.

In Nepal, from 2000-2009, 59 districts (out of 75) had the cases of rabies in dogs, cattle, buffaloes, horses, goats, pigs, and cats. Altogether 1713 animal rabies cases were found. The plain and hill areas, where 90% of population resides, were mostly infected. The sixteen districts in high hills did not have any cases. The canine breeding season seems very effective in spreading this disease due to high contact rates. February (n=250) had the highest number of cases, and May (n=89) had the least. Cattle (35.5%) were the mostly affected species, and dogs (32%) ranked second.

In Albany County (New York), 74.2% (605/815) of samples from suspected raccoons were (rabies) positive through Fluorescent Antibody test. Females accounted for 57% of the positive cases, and there was an association between gender and positive test results (p<0.01). February (93%) had the highest percentage of cases, and July had the least. Through logistic regression model, it was found that the observed raccoon's behaviors were associated with the test results. Raccoons that showed the aggression against "domestic animals" were 4 times more likely to be tested as positive rabies case (p<0.0001). The "unafraid" behavior of raccoons were 2.34 times more likely to be tested as positive rabies case (P=0.0094). Those raccoons, which were "active by day time," were 1.4 times more likely to be tested positive in the diagnosis of brain samples (p=0.0045). The "abnormal (n=141)" sign was associated with a protection from being test-positive with a risk 0.65 times (OR=0.654, CI=0.44, 0.972) as likely to be confirmed as rabid (p=0.0358). Human aggression (n=67, p=0.1177), wild animal aggression (n=12, p=0.6124), and object aggression (n=25, p=0.4036) were not significantly associated with the test results.

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CHAPTER 1: FIELD EXPERIENCE AT KANSAS STATE UNIVERSITY RABIES LABORATORY

The Kansas State University Rabies Laboratory is the primary diagnostic laboratory for rabies testing in the states of Kansas and Nebraska. In addition, the laboratory is one of the largest volume rabies serology centers in the world for both humans and animals. Each year, over 60,000 samples are handled. Laboratory diagnosticians examine brain tissues from animals suspected of rabies, and measure rabies antibodies by the Fluorescent Antibody Virus Neutralization (FAVN) test, Rapid Florescent Focus Inhibition Test (RFFIT), and Enzyme-Linked Immunosorbent Assay (ELISA); however, the ELISA method is not commonly followed.

DIAGNOSTIC TESTING FOR ANIMAL SUSPECTED OF HAVING RABIES:

I was involved in the collection of brain tissue samples received at the laboratory from animals suspected of rabies. The species were variable, and the samples were submitted from individuals and/or organizations such as county health departments. I collected brain tissues (brain stem and cerebellum) from species such as bats, skunks, raccoons, dogs, cats, and buffaloes. I am well acquainted with the methods for making impression smears on the slides, and staining them as per the Standard Operating Procedure (SOP) of the Kansas State University Rabies Laboratory. After working at the rabies laboratory, I am confident in differentiating and distinguishing between positive and negative slides of the brain samples of rabies suspected cases of various animal species.

FLOURESCENT ANTIBODY VIRUS NEUTRALIZATION (FAVN)

This test measures the response of an animal's immune system to the rabies vaccine. More specifically, it is a virus neutralization assay developed to screen animal sera by standard method to test for the presence of an adequate level of rabies anybodies following vaccination. The FAVN test is required by many rabies-free countries or regions in order for dogs and cats to qualify for a reduced quarantine period prior to entry. Regions like Hawaii, Guam, Japan, St Kitts and Nevis, Australia, New Zealand, France, and the United Kingdom require this test.

RAPID FLUORESCENT FOCI INHIBITION TEST (RFFIT):

The Rapid Fluorescent Foci Inhibition Test is a serum neutralization (inhibition) test. It measures the ability of rabies-specific antibodies to neutralize rabies virus, and prevents the virus from infecting cells. These antibodies are called rabies virus neutralizing antibodies (RVNA). In this process, serum is first diluted fivefold followed by further serial dilutions. These serum dilutions are mixed with a standard amount of live rabies virus, and are incubated so that any RVNA present will neutralize the virus. This is followed by adding tissues cells, and then the serum/virus/cells are incubated together. Whatever rabies virus is left will infect the cells which, when infected, can be differentiated by specific staining and microscopic examination. Calculation of the endpoint titer is made from the percent of virus infected cells observed on the slide. Rabies antibody level greater than or equal to 0.5 International Unit (IU)/milliliter (ml) demonstrates an adequate response to vaccination. If the level falls below this value, a booster of rabies vaccine may be recommended for people who are at frequent risk of rabies virus exposure.

The RFFIT is used in humans and animals for the purpose of screening of rabies disease or to find the end point titers; however FAVN test is used in pets to know the response of the rabies vaccine, and is required by many rabies free countries and regions to reduce the quarantine period during travel.

Apart from my specific capstone project, I tried to become acquainted with the on-going activities at the rabies laboratory, many of which require specialized knowledge. I was mostly involved in receiving of samples, processing them, data entry, and assisting in multiple areas of the laboratory. Working at the rabies laboratory exposed me to the processes necessary to handle large volumes of samples with minimum errors. By following standard operating procedures of this laboratory, I was able to handle large number of samples, and sending the reports back to the submitting individuals or organizations with due cautions and proficiencies. I believe that the experiences earned will be an asset for me, and will allow me to provide valuable input for developing the guidelines needed for the smooth running of a diagnostic laboratory in Nepal.

CHAPTER 2: RABIES – AN INTRODUCTION

Rabies is the least prioritized viral zoonotic disease in many parts of the world. It is prevalent throughout the world (with few exceptions), and is a serious health problem, mostly in developing countries (Talbi, Holmes et al. 2009). Approximately 55,000 people (mostly children) die of rabies annually (56% Asia and 44% in Africa) (WHO ; Dodet and Asian Rabies Expert 2007). Due to exposure from suspected animals, about 10 million people receive post exposure vaccine each year (Sugiyama and Ito 2007). The estimated cost of rabies is US $583.5 million, mostly born by Asian countries, where large amounts of post exposure prophylaxis are being administered (WHO).

In many developing countries, a lack of knowledge of the epidemiology of this disease limits the ability of planners to prioritize and optimize rabies control programs. The re-emergence of this disease in many previously disease free areas has been due to inconsistent rabies control programs (Davlin and Vonville 2012).

HISTORICAL PERSPECTIVES:

Historical records show that this disease has been present since antiquity. Human rabies and its transmission from the bite of mad dogs were mentioned in the Babylonian code of Eshnunna (2300 B.C.). Reports of Spanish conquistadors dying after being bitten by a vampire bat exist as early as 1514. Rabies epizootics associated with dogs were not reported until the early 18th century (Jesse D. Blanton 2011). This disease has been recognized in India and the Indian sub-continent since the Vedic period (1500-500 BC). In July 1885, Louis Pasteur successfully developed the rabies vaccine by inoculating a 9 year boy (Bourhy, Dautry-Varsat et al. 2010).

RABIES VIRUS

The rabies virus belongs to the genus *Lyssavirus* of the family *Rabdoviridae,* which are enveloped RNA viruses. The root of the genus name is attributed to the Greek goddess Lyssa, the

spirit of rage, frenzy, and madness, and the word 'rabies' itself derived from the Latin term for madness and raving (Green, Carpenter et al. 2011).

The genus is composed of rabies virus (genotype 1) and rabies related viruses, including Lagos bat (genotype 2), Makola virus (genotype 3), Duvenhage virus (genptype 4), European bat lyssaviruses 1 and 2 (genotypes 5 and 6, respectively), and Australian bat lyssavirus (genotype 7). The virus is negative-sense single stranded RNA genome of approximately 12 kb containing coding information for nucleocapsid (N), phosphoprotein (p), matrix protein, glycoprotein (G), and RNA polymerase (L) (Sugiyama and Ito 2007).

EPIDEMIOLOGY

In nature, many animal species support the maintenance and spread of rabies virus. Skunk, raccoon, and fox rabies are prevalent in many parts of the US and Canada. Jackals, bat-eared foxes, and mongooses are involved in the transmission of this deadly disease in Africa. Many bat species harbor and transmit rabies and rabies-related viruses in Australia, Africa, Central and South East Asia, Europe, and many parts of the America. Dogs are the most important reservoir in the South East Asia. Mongooses (*Herpestes* spp.), jackals (*Canis aureus*), foxes (*Vulpes bengalensis*), and wolves (*Canis lupus*) have been incriminated as wildlife reservoirs of rabies in Bangladesh, India, and Nepal (Gongal and Wright 2011).

Dogs are the most common susceptible animal species in Asia and Africa, where stray dogs are the major source of human bites and deaths, with more than 90% of human rabies transmitted from dog bites. Infected wild-animals and bats are also responsible for the transmission of this disease to humans, but the number of cases through such types of transmission is quite low in compare to canine rabies. The rabies virus is transmitted to humans through saliva contact of infected animals. A bite from a rabid animal is almost fatal if it is left untreated (Susilawathi, Darwinata et al. 2012).

PATHOGENESIS

In general, the rabies virus enters motor neurons at the neuromuscular junction. Virus particles are transported in a retrograde direction through the axons of the infected neurons at an

estimated rate of 3 mm per hour. The virus replicates in the neuronal cell body. Replication and transcription of rabies virus occurs in negri bodies, cytoplasmic inclusions of infected neurons. Rabies virus does not destroy infected cells, so neuronal transport is maintained. After infecting the brain, the virus travels to other tissues by the way of sympathetic, parasympathetic, and other cranial nerves. Upon entry in to the salivary glands, the virus replicates in mucogenic acinar cells, and is released into the saliva. In 60% or more human cases, the incubation period is between 20-90 days, and rabies develops within 6 months of exposure in 90% of human cases. In extreme cases, an incubation period of 5 years or more and as low as 5 days has been reported (Green, Carpenter et al. 2011).

RABIES STATUS IN THE CONTINENTS

Rabies is present on all the continents, and is endemic in most Asian and African countries. It is difficult to estimate the global impact of rabies by only focusing on human data since domestic animals and wild life populations are mostly excluded in the reporting of this disease. Human mortality is centered in Asia and Africa due to the scarcity of public health resources and vaccinations. In spite of the availability of rabies vaccines for more than 100 years, many developing countries are not able to provide the vaccines to large proportions of their population, who are at high risk due to close contact with dogs and wild animals. Illiteracy and poverty are other factors which synergistically increase the incidence of rabies.

ASIA

Asia is the most vulnerable continent for rabies with around 2.5 billion people at risk due to large numbers of stray dogs in close contact with rural communities. The human- dog ratio in rural and urban areas is around 14.3 and 7.4 respectively (Dodet, Goswami et al. 2008). This disease accounts for a total annual death of around 31,000. Every 20 minutes, one person dies of rabies in Asia, with children being the most commonly affected.

Dog bites are the primary source of human infection, and account for 96% of rabies cases in the South East Asian region, and thus the elimination of human rabies is dependent on the elimination of dog rabies. Based on the rabies status and incidence, the South Asian countries have been categorized into high, medium, and low endemic status. Maldives, Timor-Leste and some islands of India are historically free of rabies. Bangladesh, India and Myanmar are high

rabies endemic countries. Bhutan, Nepal and Sri Lanka are medium endemic countries. Thailand is moving towards low endemic status, but due to increasing rabies incidence, Indonesia is moving from a low endemic to medium rabies endemic status. Rabies is an emerging disease problem on many islands of Indonesia which were previously considered rabies free (Gongal and Wright 2011).

In recent years, the number of cases of human rabies in Vietnam, the Philippines, Laos, Indonesia, and China is rapidly increasing. In Vietnam, 362 case of human rabies were reported from 2007 to 2010, with the rabies epidemic during this time period occurring in 25 to 27 provinces. The lack of good vaccination plans and poorly regulated import or movement of dogs has increased the risk of canine rabies in Vietnam; the dog vaccination coverage is only 10-20% (Anh K.T.Nguyen, Satoshi Inoue et al. 2011).

In Sri Lanka, rabies is endemic, and has been identified in unique wild animals such as the palm civet- *Paradoxurus hermaphrodites* (Matsumoto, Ahmed et al. 2011). In Thailand, human rabies is transmitted from dogs. There was significant reduction of the number of human cases (0.78/100,000 to 0.12/100,000) from 1980 to 1996. Of the cases, over 90% of victims were not vaccinated, and 50% were children (<14 years). Stray and unvaccinated pet dogs were responsible for the endemics of this disease in Thailand. About 54% (13088/24332) dogs were positive for rabies from 1987to 1996 (C. Mitmoonpitak 1998).

There was a rising trend in the risk of rabies in China, the most seriously affected country after India. A total of 19,806 human rabies cases were reported in China from 1996 to 2008, with an average of 1,524 cases each year, and an incidence rate of 0.1189 per 100,000. Though there was a slight decrease in the number of reported cases of rabies in 1996, there was an increasing incidence trend due to failure of control of dog rabies, and inadequate post exposure prophylaxis to patients. A few provinces had high prevalence of rabies, and male were more than twice as frequent to be reported as a case than females. Nearly one-fourth of cases were children under 15 years old with farmers being at highest risk (63.4% cases). In the tested samples (dog's brain), the rabies virus infection risk was found to be 2.3%. The reason for increased human cases in this situation may have been due to the new standard vaccine (purified vaccine) being more

expensive (and less utilized) than the cheaper concentrated vaccine which it replaced (Song, Tang et al. 2009).

Another study showed that there was a higher incidence of deaths in urban (0.29 per 100,000 people) China than in rural (0.15 per 100,000 people). This data seems unusual compared to other parts of the world like India or Africa, where rural populations had higher incidence of this disease (Darryn L. Knobel and M. Elizabeth G. Miranda 2005). China reported approximately 3,200 human deaths from rabies in 2006. It was estimated that China administered Post Exposure Prophylaxis to 5 million people in 1998, and there are approximately 130 million dogs (Davlin and Vonville 2012).

In the Himalayan Kingdom of Bhutan, rabies was prevalent until 1990. This disease is still prevalent in the southern part (bordering India) of Bhutan, especially in dogs and cattle. And, rabies has been reported to be re-emerging in northern Bhutan; previously, this area was free of rabies. From 2006 to 2010, the disease incidence in Bhutan was 1.2 per 100,000. Attempts at mass vaccination has not been highly successful (<20% of population is vaccinated), and the number of stray dogs is increasing annually. From 1996 to 2009, a total of 814 rabies cases (cattle 55% and dog 39%) were reported in Bhutan. The laboratory confirmed 70% of submitted brain tissue samples (out of 322). Wildlife rabies has not been reported, and dogs are the major source of infection in other animals and humans (Tenzin, Dhand et al. 2011).

In India, rabies is endemic in the rural and poor communities. Apart from dogs as the main reservoir, monkeys, jackals, horses, cattle, and rodents are also contributing to the disease transmission. About 15 million people are bitten by animals annually. Most animal bites in India (91.5%) are by dogs. The incidence of animal bite is 0.174 per 100,000 populations. A person is bitten every 2 seconds, and someone dies of rabies every 30 minutes. India has approximately 25 million dogs with an estimate of dog to man ratio of 1:36. Twelve companies in India were producing the nerve tissue vaccine in required quantities for use in human (40 million milliliter) and animals (90 million milliliter) annually until 2003/04. Since then, the use of nerve tissue vaccine has been phased out and, as of 2008, replaced by modern tissue-culture vaccine. In 2004, only 39.5% of bite victims washed the wounds with soap and water, and about 46.9%

received rabies vaccination. The use of human rabies immune globulin was only 2.1% (Menezes 2008). The rabies death rate is higher in rural areas (2.49/100,000 people) than urban (0.37/100,000 people). The estimated number of bites per year from suspected rabid dogs is 893,400 in rural area and 409,400 in urban areas. The annual number of patients receiving the post exposure treatment in India is 1.07 million (Darryn L. Knobel and M. Elizabeth G. Miranda 2005).

The detail of the rabies data of many South East Asian countries has been summarized in Table 1. The highlight of the summary is India, where more than 20,000 people die of rabies (36% of cases in the world and 64% of cases in Asia). The least prioritization in control of this disease may be due to the fact that the elite are at very low risk, and poor, rural people lack the awareness and opportunity for proper prevention and treatment.

Bangladesh, the most densely populated country in the world, has a very high risk of rabies among the population. About 60% of people, experiencing an animal bite, did not wash their wounds, and about 60-70% did not get prophylaxis after exposure. About 50% of dog bite victims did not get vaccination due to poor economic conditions, lack of vaccine availability, or distrust of healthcare recommendations (Hossain, Ahmed et al. 2011).

Table 1: Distribution per year of human rabies and dog bite cases in countries of South East Asian Region (Gongal and Wright 2011):

Country	Estimated number of dog bites	Estimated number of human rabies cases	Estimated number of human cases per million of population	Source of information
Bangladesh	300,000	2,000 -2,500	13	Ministry Health and Family Welfare, Bangladesh
Bhutan	5,000	<10	3	Ministry of Health, Bhutan
DPR Korea	Not Available	Not Available	Not Available	Not Available
India	17,400,000	18,000-20,000	18	Association of prevention and control of rabies in India
Indonesia	100,000	150-300	1.3	Ministry of Health, Indonesia
Maldives	0	0	0	Not applicable
Myanmar	600,000	1000	22	Ministry of Health, Myanmar
Nepal	100,000	<100	4	Ministry of health and Population, Nepal
Sri Lanka	250,000	<60	3	Public Veterinary Service, Sri Lanka
Thailand	400,000	<25	0	Ministry of Public Health , Thailand
Timor Leste	1,000	0	0	Ministry of health, Timor Leste
South East Asia total	19, 156,000	21345-23955		

Rabies virus is enzootic throughout Africa, with the domestic dog (*Canis familiaris*) being the principal vector. Sylvatic rabies has been reported in a number of wildlife hosts, particularly in southern Africa. The estimated deaths due to rabies is about 24,000 (7,000-46,000, 95% CI) human deaths per year (David T. S. Hayman and Ashley C. Banyard 2011).

Two biotypes of rabies are recognized in the southern African sub-continent. The canid biotype cycles in carnivores of the family Canidae (dogs, jackals, and bat-eared foxes) and mongoose biotype cycles in carnivores of the family Herpestidae, mainly the yellow mongoose, (*Cynicitis penicillata)* and slender mongoose (*Galerella sanguine*). Mangoose biotype has more antigenic and genetic diversity than candid biotype (C.T. Sabeta 2003)

In 2005/06, a dog rabies outbreak in the Limpopo province of South Africa resulted in at least 20 human deaths. Massive dog vaccination campaigns significantly reduced the human rabies cases from 22 in 2006 to 3 in 2010 (Sabeta, Mkhize et al. 2011).

In Ghana, rabies is present in the dog population for many decades, and disease control methods (vaccination and removal of stray dogs) have not been successful. During 2000 to 2004, 123 clinically confirmed cases were recorded (David T. S. Hayman and Ashley C. Banyard 2011).

In Zimbabwe, the domestic dogs (*Canis familiaris*) accounted for approximately 45% of all confirmed rabies cases. Dog rabies was prevalent in rural areas, where 71.3% of the country's dog population was found. Approximately 25% of all confirmed rabies cases in Zimbabwe were diagnosed in jackals (*Canis adustus* and *Canis mesomelas*) (C.T. Sabeta 2003).

One study in Africa showed that almost 70% of human rabies cases were in children and young adults below 20 years of age, and out of all positive cases, two third was male. A small number of human cases have also been due to exposure to rabid cats and wildlife. About 25% of the confirmed human rabies cases in the South Africa were attributed to mongoose exposures

(Weyer, Szmyd-Potapczuk et al. 2011). The estimated human mortality caused by canine rabies in Africa and Asia has been summarized in table 2.

Table 2: Estimated human mortality caused by canine rabies in Africa and Asia (Darryn L. Knobel and M. Elizabeth G. Miranda 2005)

| Model Output | Asia | | | | | | | |
| | India | | China | | Other Asia | | Africa | |
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
Total population (millions)	284.7	732.2	459.1	816.1	295.7	525.4	294.2	498.1
Populations at risk (millions)	284.7	710.4	459.1	498.3	295.7	409.1	294.2	340.1
No. bites from suspected rabid dogs (thousands)	409.4	893.4	660.1	626.7	425.2	524.5	374.3	427.8
No. of rabies deaths (a)	1058	18201	1324	1257	853	8135	5886	17937
No. deaths/100,000 people	0.37	2.49	0.29	0.15	0.29	1.55	2.00	3.60
No. sub regional deaths (b)	19,713 (4,192-39,733)		2336 (65-5,049)		9489 (2,281-19,503)			
Total no. deaths	31,539 (8149-61,425)						23,705 (6,903-45,932)	
Total no. of deaths	55, 270 (23, 910- 93, 057)							
Overall no. deaths/100 000 people	1.38 (0.60-2.33)							
Predicted deaths in the absence of any post-exposure	327, 160 (166, 904-525, 427)							

(a) Rabies deaths are the means of output probability distributions calculated independently may therefore not sum exactly

(b) Figures in parenthesis are the 5th and 95th percentiles of output probability distributions.

EUROPE

Epidemics of rabies have been recorded in Europe over the past centuries. Dog were main host and primary transmitter to humans. The current epidemic in Central Europe is thought to have started in Poland in 1939. Foxes play a dominant role in the transmission and maintenance of the diseases, although other mammals (dogs, skunks, raccoons, wolves and bats) are also important (Roy M Anderson, Helen C Jackson et al. 1981). From 1990 to 2010, a total of 22 human deaths from rabies were reported in Europe; mainly in travelers from rabies-endemic countries (Malerczyk, Detora et al. 2011).

Until 1985, bat rabies was endemic only in the northeastern part of Europe (Northern Germany, Poland, former Soviet Union, Turkey, and Yugoslavia). Since 1985, Denmark, the Netherlands, Germany (Southwest), Spain, and east of France have reported cases of bat rabies (Herve Bourhy 1992).

The red fox (*vulpes vulpes*) is currently the main terrestrial wildlife rabies vector in Europe. However, recently the raccoon dog (*Nyctereutes procyonoides*), an invasive species originating from East Asia, has become increasingly important as a secondary host. especially in the Baltic States (Alexander Singer 2009).

LATIN AMERICA

In 2004 and 2005, fatal cases of human rabies transmitted by vampire bats were 46 and 55 respectively. In 2004, the number of human rabies cases transmitted by wildlife (mostly vampire bats) exceeded the number of cases transmitted by dogs. Out of 55 vampire bats cases in 2005, 41 cases were in Brazil, 7 in Peru, 3 in Colombia, 2 in Ecuador, and 1 in Bolivia (Maria Cristina Schneider, Hugo Tamayo et al. 2009).

During 1998-2007, one or more cases of human rabies transmitted by dogs had been reported in 10 countries: Bolivia, Brazil, Colombia, Cuba, El Salvador, Guatemala, Haiti, Mexico, Peru and Venezuela. But cases of canine and human rabies have been reduced by nearly 90% over the past 20 years (Schneider, Aguilera et al. 2011).

From 1990 to 2003, the numbers of human rabies cases were decreased from 251 to 35 (86% reduction). Dog exposure accounted for 65% of all cases. One thousand one hundred thirty one cases of canine rabies were reported in 2003 in 12 countries of Latin America. Forty two million dogs were vaccinated annually in Latin America; most of them are in Brazil (75%). Nearly 1 million individuals at risk of rabies infection were treated annually. On average, 73,752 canine samples were processed for rabies diagnosis per year in South America. The mean number of samples submitted for analysis was 0.05% of the region's estimated canine population (Schneider, Belotto et al. 2007).

NORTH AMERICA

During 2010, 48 states and Puerto Rico reported 6,154 rabid animals and 2 human rabies cases to the Center for the Disease Control (CDC), representing an 8% decrease from 6,690 rabid animals, and 4 human cases reported in 2009. Hawaii and Mississippi did not report any laboratory-confirmed rabid animals during 2010. Approximately 92% of reported rabid animals were wildlife (36.5% raccoons, 23.%% skunks, 23.2% bats, 6.9% foxes, 4.9% cats,1.1% cattle and 1.1% dogs). In compared to 2009, the number of reported rabid animals decreased across all animal types with exception of a 1% increase in the number of reported rabid cats. In 2010, two cases of human rabies (due to bite of bats) were reported from Louisiana and Wisconsin (Jesse D. Blanton 2011).

Approximately 17% of *Eptesicus fuscus (E. fuscus)* and 16% of the Mexican free-tailed bats, *Tadarida brasiliensis* submitted to diagnostic laboratories in Colorado and Texas were rabies positive. During 1988-2007, 3.3 % (1241/36506) *E. fuscus* submitted to the New York State Diagnostic Laboratory were found to be rabid (April Davis 2012).

Since 1950, 55 human cases of bat-variant rabies were reported in the United States and 6 cases have been reported in Canada. Over all since 1950, an incidence rate of bat rabies in humans of 3.9 cases/billion person-years has been reported for both the United States and Canada. In Mexico, 2-3 human cases of bat rabies were identified per year since 2000 although the trend as a proportion of all human cases of rabies has been increasing in recent years (De Serres, Dallaire et al. 2008).

CHAPTER 3: STATUS OF RABIES IN NEPAL

INTRODUCTION

Nepal, an area of 147,181 square kilometers, has diversified topography, ecology, climate, and culture. It is surrounded by India in the south, east and west, and Tibet of China in the north. The northern border is mostly covered with the snow and mountains, and serves as a natural barrier to the transmission of rabies into Nepal from that direction. But, the rapid communication and open border with India has increased the threat of transmission of many infectious diseases (along with rabies) between India and Nepal.

Nepal has a total population of 29,331,000; the majority of people are involved in agriculture as their way of life. Direct contact of people and animals has a strong positive impact on the risk of transmitting the zoonotic diseases to humans. Children are directly associated with animals (domestic and pets), and they are always a high risk group for rabies exposure in poor rural communities. The low literacy rate is a major factor responsible for the failure of many awareness programs launched by the government and non-government organizations. Though health issues in Nepal are addressed better than in the past, the zoonotic diseases including rabies are still under reported and less prioritize.

Unfenced forests and frequent contact between wild and domesticated animals or pets always create an increased threat for spread of rabies from wild animals. In Nepal, stray dogs are the prime reservoirs and vectors for rabies transmission to humans and animals.

Kathmandu, a city with over 3 millions human populations, has a large population of free roaming dogs that are known to transmit rabies virus. The majority of human rabies cases in Nepal result from canine exposures.

Rabies in Nepal is maintained by the two interrelated cycles viz. sylvatic and urban. The sylvatic cycle is mainly maintained by foxes and jackals. Also, rabies transmission by vampire bats (*Desmodus rotundus*) is an important public health concern. However, the major cause of rabies in both human and livestock is due to the urban cycle (dog bite).

Annually more than 30,000 people in Nepal receive post exposure treatments. More than 94% of reported cases are due to dog bites, 4% due to jackals and the rest due to mongooses, cats, and other animal bites. Ninety-six percent of human rabies deaths in Nepal result from canine exposures (Prativa Pandey 2002). The risk of rabies in the international travelers in Nepal was 1 in 6,000 travelers (Ranney, Partridge et al. 2006).

World Health Organization (WHO) surveyed that 160 to 170 human rabies cases were clinically confirmed, and approximately 35,000 persons required treatment after potential exposure to rabies in Nepal in 1999 (Kato 2003).

Monkeys are also an animal population of interest in that they are responsible for many human bites, particularly in Kathmandu. Many children and tourists have been found to be bitten by monkeys near certain temples in Kathmandu valley. But the risk rate of acquiring rabies from these monkeys has not been studied; but it is believed to be an important potential method of transmission due to close contact between monkeys and stray dogs. It has been demonstrated that monkeys can be responsible for transmitting rabies in some cases, for example a 10 year Australian boy in India (Prativa Pandey 2002).

STREET DOGS IN NEPAL

We did not find any estimated street dog population for the entire country; however, it has been estimated that in Kathmandu, there are more than 20,000 street dogs. These dogs are injured from collisions with cars, are starved, and have open sores with maggot infections. Some stray dogs carry rabies, which pose a significant risk to people, particularly to children, who often play in the streets. The street dogs are most likely to bite and transmit rabies during the breeding season.

Every year, around 200 people die of rabies (however WHO reports less than 100 cases annually), and 16,000 are treated for dog bites (many cases are under reported) (Table 1). The Kathmandu city government used to poison more than 10,000 street dogs each year with

strychnine in an attempt to control dog population. But now, many organizations are taking responsibility to neuter (spaying) the street dogs as a way of controlling their population.

DISEASE SURVEILLANCE:

In Nepal, animal rabies surveillance is passive. The Central Veterinary Epidemiological Unit (CVEU) at the Department of Livestock Services (DLS) of Nepal receives a monthly Animal Disease Epidemiological Report in a specified format from all 75 District Livestock Service Offices (DLSO). All DLSOs have a network in Village Development Committee (lowest unit of bureaucracy) to deliver animal health services. The animal health technicians collect animal health and diseases data using a prescribed format, and send it to the respective DLSOs. DLSOs review the information, compile it, and send it to the Central Veterinary Epidemiologic Unit (CVEU) of Nepal. The CVEU assembles the data from all the districts, analyzes it, and publishes a report in a periodical bulletin or other publications.

If there are clinical cases of rabies in animals, DLSO will be responsible for bringing the sample (brain) to the Central Veterinary Laboratory (CVL). The CVL processes and diagnoses the sample, and sends the reports back to the DLSO and CVEU. Rabies diagnosis is free of cost, but the diagnostic technique is available only at the Central Veterinary Laboratory of Nepal.

NETWORKING OF VETERINARY SERVICES

There are 75 DLSOs, under which more than a thousand service centers (and sub-centers) work in the animal health sector in rural areas. Regional Veterinary Laboratory (RVL) and Regional Livestock Service Directorate (administrative) have authority to supervise all the DLSOs in that particular region. The Central Veterinary Laboratory in coordination with the Directorate of Animal Health and Regional Veterinary Laboratory (RVL) takes the lead role in the surveillance and control of animal diseases. Unfortunately, RVLs do not have technical facilities to cope effectively with rabies. The Office of Veterinary Public Health is taking a major role in spreading the awareness campaign, neutering stray dogs, vaccinating dogs and other preventive roles. The private veterinary practitioners are involved in the vaccination of dogs. Other animals are not vaccinated unless they are exposed to a dog or wild animal bite.

Though there are extensive health services for animals and humans, the low literacy rate of the country is always a challenge for controlling this disease. Dog bites are generally ignored, and that lack of vigilance often costs exposed people their life. Close contact between unvaccinated dogs and humans always increases people's (especially children's) risk.

The dog neutering programs are generally centered in the urban area. This has definite benefits because in Kathmandu, the stray dog density in 1997 was 2,930 dogs per square kilometers, and the ratio of stray dog to humans was 1:4.7 (Kato 2003). However, there is essentially no effective control mechanism for rabies in villages, where the largest proportion of the population of the country lives, and in villages, the population has close contact with animals due to their dependency on agriculture. Figure 1 (adapted from WHO/Nepal Website) shows the distribution of rabies risk in humans in the different districts of Nepal.

Figure 1: Distribution of human rabies in Nepal

METHODOLOGY

We had access to ten years (2000-2009) historic animal rabies data of Nepal, and based on these data, we tried to find the status of animal rabies in Nepal considering the geography, species of animals, seasonality of disease occurrence. The data has been collected by the District Livestock Services of Nepal as passive surveillance of diseases, and is entirely based on the clinical examination by a qualified veterinarians and technicians. Laboratory confirmations of these cases were not found. We used the Microsoft Excel program and descriptive statistical tools to describe the animal rabies status in Nepal. The aim of this study was to know the distribution of animal rabies cases in the country.

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RESULTS AND DISCUSSION

STATUS OF ANIMAL RABIES IN NEPAL

During 2000-2009, there were 1,713 reported animal rabies cases in different districts of the country. The highest number of cases was found in Jhapa (n=149), which is one of the most densely populated districts in Nepal, and shares the borders with two states (Bihar in south and west Bengal in East) of India, and also has dense forests with high wildlife populations. This district also has an unmanaged population of Bhutanese refugees, who are living with their livestock and pets (dogs) in the region, which increases the risk of the disease in this district. But in 2008 and 2009, no cases were recorded in this district.

In Rolpa, one of the districts in the hill region, there were 143 cases. Nawalparasi, the other district of the plain adjoining with India, had 126 cases during this period. This district is also densely populated, and has large numbers of cattle (milk producing area), and shares forests and national parks (Chitwan Wildlife National Park).

The 16 districts including hills and mountains (Argakhachi, bajhang, bajura, Dailekh, Dolakha, Dolpa, Gulmi, Mugu, Mustang, Miyagdi, Nuwakot, Parbat, Ramechhap, Rasuwa, Sindhuliplanchowk, solukhumbu) did not have any cases recorded during this period.

The mean number of cases in the entire districts of Nepal was 171 (Standard deviation 92), median and range of the number of cases were 183 and 297 respectively.

Figure 2: Cases and number of districts

25
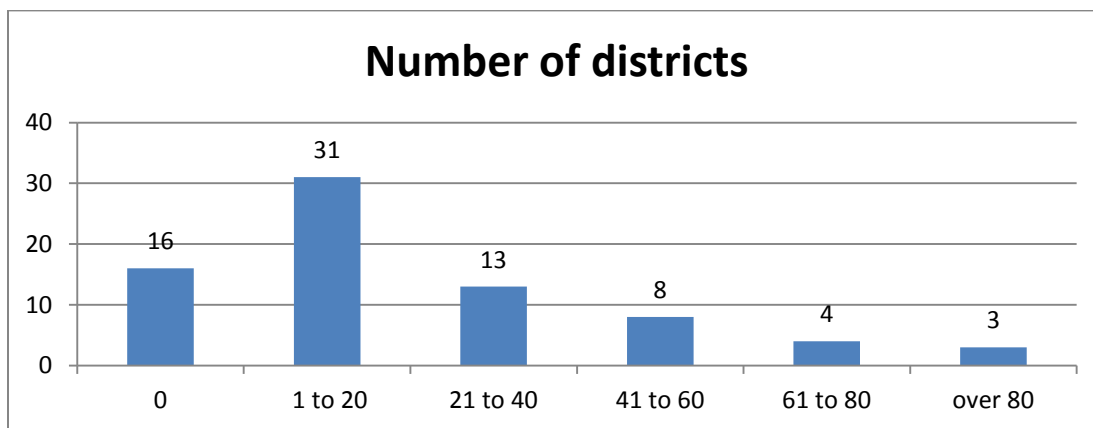
Thirty one districts had the animal rabies cases at least 1 to 20. Only 3 districts had more than 80 cases in this period, and 16 districts did not record any cases (figure 2).

RABIES IN VARIOUS ANIMAL SPECIES

We found that buffaloes, dogs, cattle, goat, horses, pigs and cats were infected with rabies during 2000-2009. The highest number of clinically diagnosed rabies cases (n=608) were found in cattle, which were 35.5% of all the cases recorded during this period. About 32% (553/1713) of the cases were dogs, and 22% (384/1713) were buffaloes. 7% (132/1713) goats, 1.3% (23/1713) pigs, 0.5% (8/1713) horses, and 0.3% (5/1713) of the cases were sheep. The finding that few dog's cases were reported than cattle cases may be due to under-reporting of dog cases since they are strays and not the responsibility of any person.

SEASONAL TRENDS OF ANIMALS RABIES

The breeding behaviors of dogs are controlled by the seasons. In Nepal, breeding season of dogs starts before the onset of winter (October, November). In this season, dogs have higher contact rates, and they are often seen in groups. So the chances of transmission of the disease from one dog to another become high. In our study, we found the highest number of cases in February (n=250), which may be due to increase in disease transmission during the breeding season, with the appearance of disease after an expected incubation period. The increase in the cases of rabies in June may be due to increased contact of wild animals and domesticated grazing animals in the months of summer (May/June), when animals are left on free range grazing.

Figure 3: Seasonal trends of animal rabies in Nepal
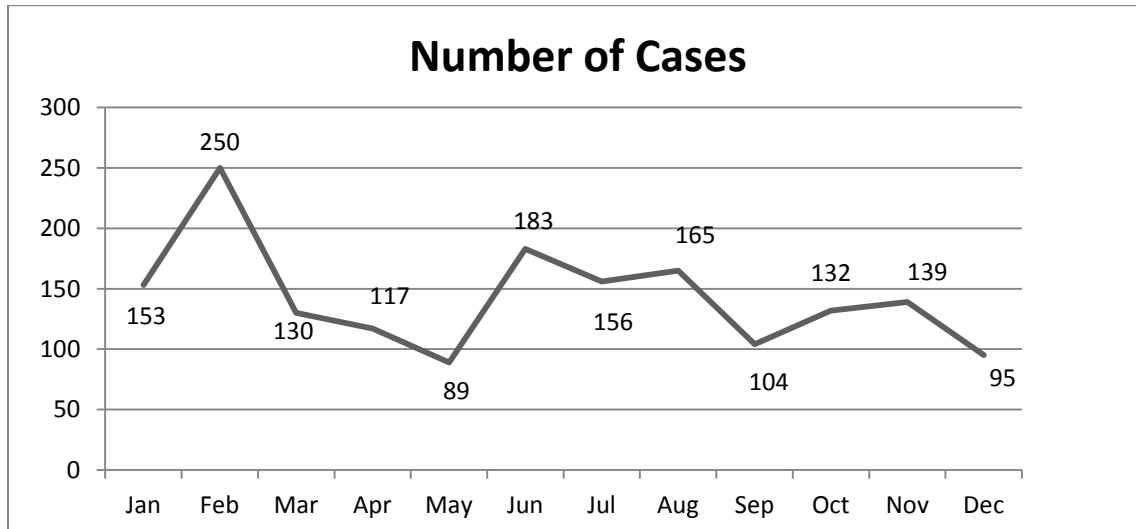
As in figure 3, we found the highest number of cases in February (n=250), and lowest numbers of cases were recorded in May (n=89). Two peaks, one in February and other in June (n=183) were seen, which may indicate the effect of season on the number of positive cases.

TREND OF ANIMAL IN ECOZONES

Though Nepal is a small country in geography, it has wide range of diversity in the climate and topography. The southern plain covers 17% of the land with an altitude range from 100m to 300m. Because of fertile land, it has 48% of the country's population. Some of the best wildlife reserves, and national parks are in this region. The middle hill, also called as Mahabharata range, accounts for 65% of the total area of the country, and altitude varies from 500-3000 meters above the sea level. The Himalayan or mountain range covers 16% of the total land of country, and ranges in altitude from 2,500 to 8,848 meter from the seas level. This range includes 8 to 14 of the highest peaks in the world, having altitudes more than 8,000 meters.

Figure 4: Distribution of animal rabies cases (eco-zones)
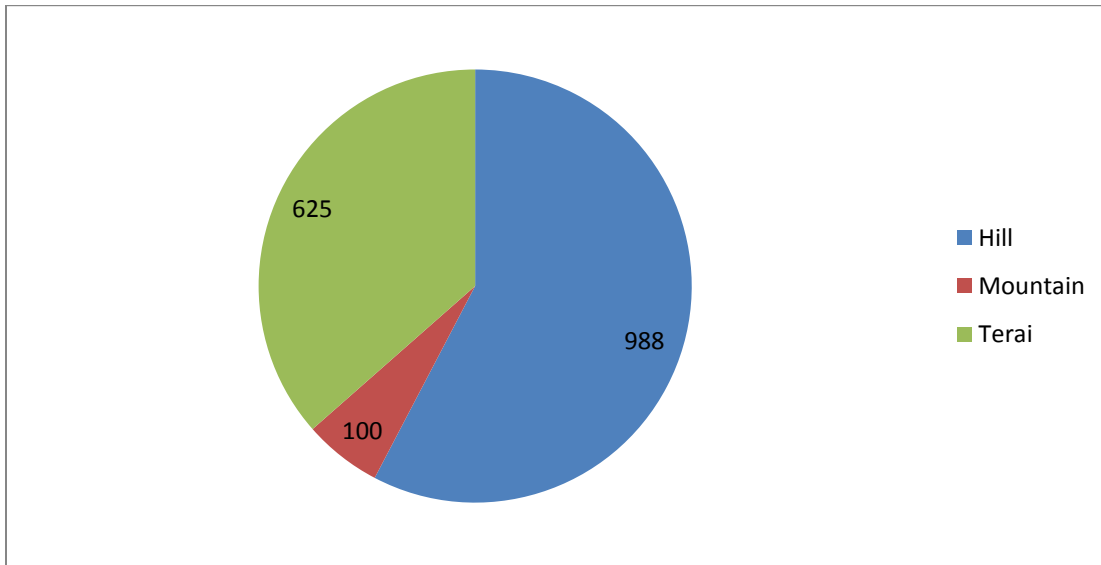
We found that the hilly regions had the most reported rabies cases (n=988, 58% of all cases in Nepal) during 200-2009, which is different than the distribution of human cases (human cases highest reported in terai as in figure 1). There were 625 cases (36%) in the southern plain region, and mountain region had the least cases (n=100, 6%). The higher number of cases in hills and terai may be due to higher density of animal population (along with dog), and grazing lands near the forests make the animals of this region at higher risk for this disease. Wild animals like jackals and foxes, which reside in or near the forest frequently come in contact with grazing animals, which may help explain the fact that 95% of rabies cases in Nepal were reported from the hills and terai.

CONCLUSION

In spite of rabies continuing to have a tremendous impact on human health, particularly children, risk of human rabies has been reported to be decreasing in recent years. While analyzing the 10 years of historic data of Nepal, it can be concluded that this deadly disease is almost evenly spread throughout the country, and cases have been reported throughout the year. The intimate interaction of animals and humans will always poses a serious threat to human health, and there continues to be a need for better public awareness about the seriousness of rabies, the availability of vaccines, the importance of post exposure treatment, and control of

stray dogs. Decreasing contacts between wildlife and domesticated animals should have some affect in decreasing the cases of rabies in the future.

CHAPTER 4: RACCOON RABIES IN ALBANY COUNTY, NEW YORK IN 1993

BACKGROUND

Raccoon rabies is prevalent in the eastern United States, and poses a serious threat to humans. It has been found in New York State since 1990 (Recuenco, Eidson et al. 2007). Prior 1977, raccoon (*Procyon lotor*) rabies was confined to the southern US. Translocations led to the emergence of this rabies variant in the mid-Atlantic region, followed by spread north to northeast Ohio and Ontario, and New Brunswick, Canada. Now, it is enzootic in all of the eastern coastal states as well as in Alabama, Ohio, Pennsylvania, Tennessee, Vermont, and West Virginia (Jesse D. Blanton 2008).

Until the mid 20th century, dogs were the primary source of rabies in the US (Jesse D. Blanton 2011). Before 1990, rabies infections in New York were attributed to red foxes and bat variants of the virus. After 1993, rabies testing indicated that the red fox variant no longer existed in the state, instead, a raccoon rabies variant had moved into New York State from Pennsylvania in 1990 (Hwa-Gan H. Chang and Robert Rudd 2002).

In the North America, variants of rabies virus are maintained in the wild by several terrestrial carnivores species, including raccoons, skunks, and bats. Each genetically distinct variants of the virus in mammalian species occurs in geographical discrete areas, and is strongly associated with its reservoir species. Within each area, a spillover of rabies into other species occurs, especially during epizootics. As a result of spillover, a variant may eventually adapt to a secondary species, which may begin to serve as alternative reservoir species (Marta A. Guerra and John W. Krebs 2003).

Raccoon rabies, in the United States, has been a public health concern since the original findings of raccoon variants of rabies in 1940s in Florida (James E. Childs and Charles E. Rupprecht 2001). Within the United States, about 90% of rabies cases reported to the Center for Disease Control and Prevention (CDC) occur in wildlife. The most prevalent rabies variant is associated with raccoons (Robert B. Puskas 2010).

In New York State (NYS), from 1990 to 2004, this disease has been confirmed by the laboratory in 14,892 terrestrial animals, 74% (10,980) of these cases were raccoons. Persistence of the raccoon-rabies endemic in NYS for many years indicates that there are probably many favorable factors associated with maintaining the infection. Raccoons live close to forests landscapes, and also adapt to living in urban setting where food is easily and abundantly available. NYS includes diverse landscapes, elevations, and human population densities. The closeness of human residence near the forests is helpful in increasing the rabies cases, as raccoon can live in the forest and acquire food from the nearby houses. Contact between forest and human housing also increases contact between raccoons and pets. High elevations, and high water sheds are protective against raccoon rabies; such type of lands prevent easy movement of raccoons. Sometimes, water-shed areas, large rivers, and mountains become natural barriers for raccoon movement and rabies transmission. Major roads are also barrier for raccoon rabies transmission (Recuenco, Eidson et al. 2008).

During 1993-2003, 127 cases of raccoons variant rabies were reported in raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) in Ontario, Canada (Rick Rosatte, Kim Bennett et al. 2006). In 2009, there were 6,690 cases of animal rabies in 49 States of the US and Puerto Rico. About 92.5% of cases were in wildlife, 7.5% were in domestic animals. Over one third (34.8%) of animals rabies cases were in raccoons (Green, Carpenter et al. 2011).

Raccoon population biology in the northern Atlantic states is characterized by a birth season in the winter, the emergence of young in the spring, a period of growth during the summer when young animals stay with their mothers, and a period during the fall when juvenile disperse away from their natal territories (Colin A. Russell 2006). A number of mechanisms have been proposed connecting climate cycles to seasonal outbreaks of disease, including change in host physiology, and seasonal increase in contact between hosts which may be associated with migration and breeding (Duke-Sylvester, Bolzoni et al. 2011).

The fluctuations in contact rates due to breeding behavior and dispersal of the young may cause a characteristic seasonal variation in the incidence of rabies in wild animals including

raccoons. The temporal trends of rabies in the wildlife are influenced by population dynamics and changing number of susceptible animals (Mary E. Torrence 1992).

The increase in the number of rabies in spring may be related to the emergence of susceptible yearlings and juveniles (Xingtai Wang and Evan Caten 2009). In Ontario, Canada, the majority of rabid raccoons were reported during the fall, winter, and spring, suggesting a relationship between raccoon behavioral activities such as breeding and timing of rabies outbreaks (Rick Rosatte, Kim Bennett et al. 2006).

METHODOLOGY

DATA COLLECTION

The data was collected as active surveillance of raccoon rabies in the Albany county of New York from November 1992 to December 1993. The Department of Environmental Conservation, Wildlife Pathology unit was involved in the surveillance. They responded to request for assistance from the public in regard to dead or sick wildlife. They prepared samples for submission to the rabies laboratory (Wadsworth Center). During the specified period, 815 raccoon rabies samples of different age, sex and gender were collected and submitted to the laboratory for diagnosis. Various signs and behaviors of the raccoon were documented. All the samples brought to the laboratory were tested with Direct Fluorescent Antibody test using rabies antigen. We analyzed the data based on raccoon attributes like age, sex, gender, seasonality, and behaviors to examine the role of such attributes on the likelihood of test results. These attributes would be helpful for people and professionals while handling the rabies suspected cases of raccoons.

Apart from other attributes of raccoons, we were interested to know the association of observed behaviors of raccoons with test results. We used the logistic regression model to test the association of raccoon behaviors with test results. We also looked at the association between exposure type (bite or salivary contact) on the likelihood of human exposure.

STATISTICAL TOOLS

To analyze these data, we used descriptive statistics and logistic regression model or chi-square test where appropriate.

LOGISTIC REGRESSION

Logistic regression is a type of regression analysis used for predicting the outcome of a categorical variable based on one or more predictor variables. Logistic regression can be bi-or multinomial. Binary logistic regression refers to the instance in which the criterion can take only two possible outcomes. Generally the criterion is coded as "0" and "1" in binary logistic regression as it leads to the most straightforward interpretation. Logistic regression is used to predict the odds of being a case based on the predictors. The odds are defined as the probability of a case compared to the probability of being a non-case. The odd ratio is the primary measure of effect size in logistic regression. An odds ratio of one indicates that the odds of a case outcome are equally likely for both groups under comparison. The further the odds deviate from one, the stronger the relationship, the odds ratio has a floor of zero but no ceiling (upper limit); theoretically the odd ratio can increase infinitely.

RESULT AND DISCUSSION

Out of raccoon brain samples (n=815) submitted to the laboratory, 74.2% (605/815) were (rabies) positive through Fluorescent Antibody test. This data indicates a slightly higher positive percentage than recent studies. The high percentage of cases may be due to the active surveillance of the disease in this study. In addition, in this study researchers intentionally looked for suspected cases, and the study year (1993) was a high year for rabies incidence in New York because the disease was new to the area, vigilance as maintained.

From 1984 to 1989, out of 3,256 raccoons submitted for rabies testing, 1,053 (32.3%) were confirmed to be positive (Mary E. Torrence 1992). In 2010, 36.5% (2246/6154) raccoons were positive in a study conducted in 48 states of the US and Puerto Rico (Jesse D. Blanton 2011). Another study conducted in 2007 showed that 36.6% (2659/7258) of raccoons were positive. In 1992, the state of New York reported 1761 cases (79% raccoons) rabies (Krebs JW 1994).

The current data shows tremendous decrease in raccoon rabies in New York State, but in 1990s there is high prevalence of this disease, which is also supported by this study. In a study carried out during 1997-2003 in various counties of New York (including Albany) showed that out of 4,871 terrestrial rabies cases, 63.7% were raccoons (Recuenco, Eidson et al. 2007).

In Ontario, Canada, during 1999-2003, raccoons accounted for 98% (125/127) of the reported cases; with behaviors including aggression, fighting with dogs, ataxia, vocalizations, and appearance of being sick (Rick Rosatte, Kim Bennett et al. 2006).

GENDER

Out of all the suspected submissions, 52% (427/815) were females and 38% (307/815) were males. Of the test-positive submissions 57% were females (344/605) and 33% (200/605) were males, and 10% (61/605) were submitted without a gender being identified (Table 3).

Table 3: Raccoon cases based on gender and test results:

Gender	Positive	Negative	Total
Male	200 (65%)	107 (35%)	307 (38%)
Female	344 (80%)	83 (19%)	427 (52%)
Unidentified sex	61 (75%)	20 (25%)	81 (10%)
Total	605 (74%)	210 (26%)	815

In this data set of raccoons with an identified gender, females accounted for 57% of the positive cases providing evidence for an association between gender and positive test result (p<0.01). As male raccoons live isolated lives in their own territory, and females live in groups, this association may be real. The young live in close association with females, so an increased risk of transmission of rabies to females mostly looks justifiable based on this data.

In Ontario, Canada, 55% (46/83) of test-positive raccoons were adult females and 45% (37/83) were males (Rick Rosatte, Kim Bennett et al. 2006).

In this data set, we divided the raccoon population into young (up to1 year) and adult (more than 1 year). Adult raccoons (n=673) accounted for 83% (673/815) of all the submission, and 92% of them were positive. While young raccoons (n=142) accounted 17% (142/815) of all the submission, and 8% (49/605) were test positive (Table 4). I did not find any supporting data reflecting the association of raccoon's age and rabies test results.

Table 4: Age-wise distribution of raccoons and test results

Age group	Positive	Negative	Total
Young Raccoons (0-1 year)	49 (8%)	93	142 (17%)
Adult Raccoons (>1 year)	556 (92%)	117	673 (83%)
Total	605 (74%)	210	815

The data indicated that there was association between rabies cases and age (p<0.01).

MONTHS AND SEASONALITY

The month of June had highest number (n=154) of submissions, of which 68% (105/154) were positive, and the least number of positive cases were in December (n=11, positive= 9). But the percentage of positive findings, out of all the submission in a particular month, was highest in February, indicating that a raccoon bite in February might have more chance of transmitting the disease. There were two months (February 93% and September 88%), which had peak in the percentage of positive cases in a 12 months analysis. The other study in Ontario, Canada had peaks in December and January (Rick Rosatte, Kim Bennett et al. 2006).

In New York, raccoon mating starts in late January or the first half of February (W. J. Hamiton). As mating increases the contact rate between raccoons, this time period may be favorable for the transmission of diseases from rabid raccoons to susceptible ones.

A study carried out in Virginia showed that the bimodal peak in seasonal rabies activity probably reflects the underlying biological phenomena. The rise in cases early in the year may correlate with the breeding season (January to March), which increases the contact rates and

aggressions. The second rise in the rabies may be related to the dispersion of young to adult population in the autumn (Mary E. Torrence 1992).

In this analysis, the percentage of submissions that tested positive ranged from 67% (July) to 93% (February), with range 25%, mean 77.77%, and media 75.9%.

Table 5: Number of raccoon cases based on months

Months	Positive (%)	Negative (%)	Total
January	21 (78%)	6 (22%)	27
February	26 (93%)	2 (7%)	28
March	55 (74%)	19(26%)	74
April	78 (75%)	26 (25%)	104
May	93 (76%)	29 (24%)	122
June	105 (68%)	49 (32%)	154
July	87 (67%)	42 (33%)	129
August	59 (76%)	19 (24%)	78
September	37 (88%)	5 (12%)	42
October	20 (69%)	9 (31%)	29
November	13 (87%)	2 (13%)	15
December	9 (82%)	2 (18%)	11
Total	603 (74%)	210 (26%)	813

(1 case had no information about date, and other was of 1992)

In Ontario, Canada, 63% (79/125) of the cases of raccoon strain rabies occurred during the breeding/parturition periods (November to May). Only 22% (28/125) of the cases occurred from June and August, and 14% in September and October (Rick Rosatte, Kim Bennett et al. 2006).

Figure 5: Raccoons cases and test results based on months:
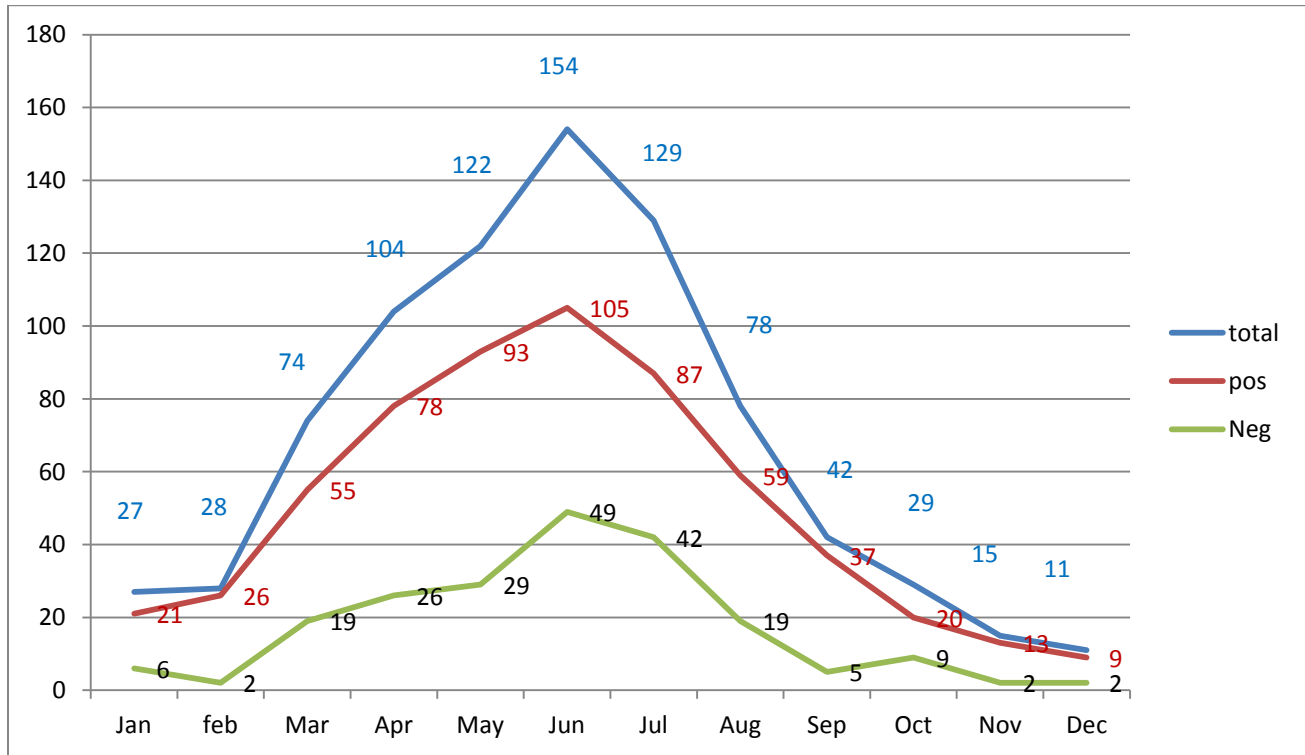
Though the number of suspected rabid raccoons (n=509) were mostly found in the months of summer (April, May, June, July), the percentage of positive cases were least in this season (71%); however, the spring had the least number of suspected rabid raccoons (n=140), but had highest percentage of positive cases (79%). In the fall (Aug-Nov), the total number of cases were 164, and 78% of them were positive.
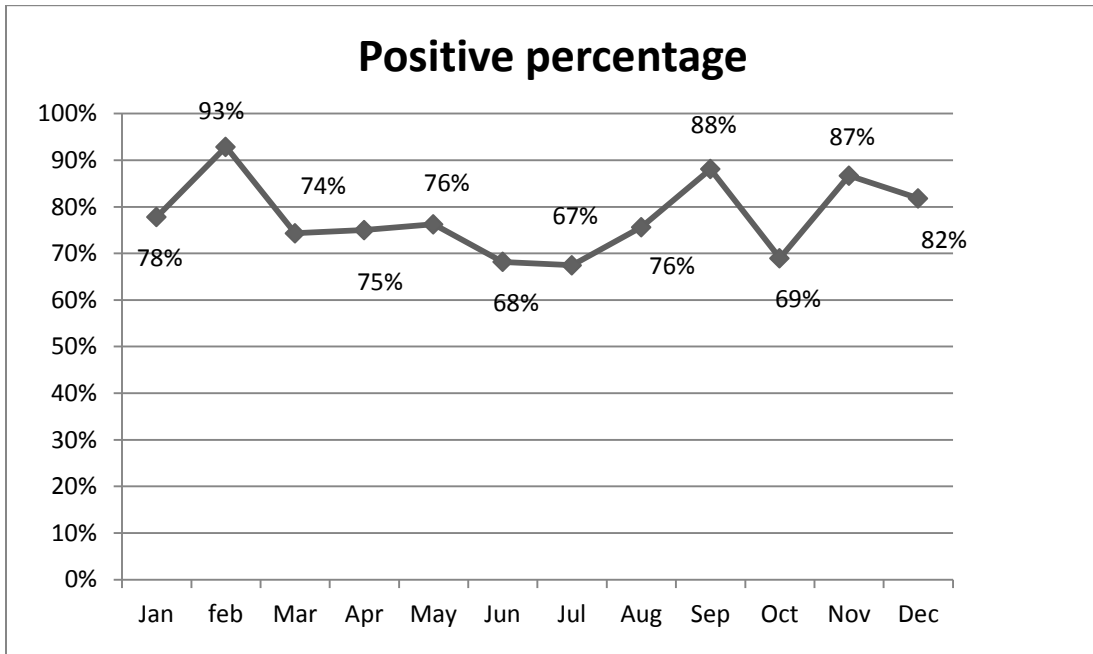
Figure 6: Percentage of raccoon cases (month-wise)

Table 6: Raccoons cases and test results in different seasons

Seasons	Positive	Negative	Total
Fall (Aug, Sep, Oct, Nov)	129 (78%)	35 (22%)	164
Spring/Winter (Dec, Jan, Feb, March)	111 (79%)	29 (21%)	140
Summer (Apr, May Jun, July)	363 (71%)	146 (29%)	509
Total	603	210	813

The data shows that there were high numbers of raccoons suspected in the summer, which may be due to increased raccoon activity due to favorable weather. It might be that the surveillance activities were more active during this period due to better weather conditions.

The percentages of positive cases were more than 70% in all seasons. But with the increase in the number of suspected cases, the number of positive cases also increase (figure 8).

One study carried out in West Virginia showed that the proportions of rabies-positive specimens was highest in April, and raccoon rabies reached second peak in the month of August (Xiaoyue Ma 2010), which was different than our findings.

A study carried out in Massachusetts showed that positive rabies specimens were most frequently detected in the spring and fall. The summer had the highest proportion of submission, but the least percentages of positive cases (Xingtai Wang and Evan Caten 2009). This study also supports our findings that we had higher number of submission in the summer, with less percentage of positive cases, and fall and spring had less number of cases, but higher positive percentages of rabid raccoons.

Fall and winter may be associated with fewer encounters with potentially rabid raccoons, but a higher likelihood of an encounter being with a test-positive raccoon.

ASSOCIATION OF RACCOON OBSERVED BEHAVIOURS AND TEST RESULTS

We analyzed different behaviors of raccoons to test their association with the test results by the use of Logistic Regression Model (SAS Software). We found that some observed behaviors of raccoons were highly associated with the test results. The associations of different reported raccoon behaviors (aggression toward humans, aggression toward domestic animals, object aggression, activity during the day, appearing unafraid and other abnormality) were tested against the likelihood of a test results. The results have been summarized in the table 7.

Table 7: Analysis of maximum likelihood and odd ratio estimates

Parameter	Point Estimate (OR)	95% Confidence Limits		Standard Error (SE)	Probability (P-value)
Domestic Animal Aggression	4.121	2.150	7.897	0.3318	<0.0001
Unafraid	2.340	1.232	4.447	0.3275	0.0094
Activity by Day	1.466	1.009	2.130	0.1905	0.045
Abnormal	0.654	0.440	0.972	0.2020	0.0358
Human Aggression	1.694	0.875	3.277	0.3367	0.1177
Wild Animal Aggression	0.727	0.211	2.499	0.6306	0.6124
Object Aggression	0.687	0.285	1.658	0.4496	0.4036

We found that raccoons which showed "aggression against domestic animals (n=121)," were 4 times (OR=4.121, CI= 2.150, 7.897) more likely to be tested as positive rabies case (p<0.0001) than raccoons that did not show this behavior. The "unafraid (n=81)" behavior of raccoons was associated with 2.34 times (OR= 2.340, CI= 1.232, 4.447) more likely to be tested positive rabies case (P=0.0094).

Those raccoons, which were "active by day time (n=226)," were 1.4 (OR=1.466, CI= 1.009, 2.130) times more likely to be tested positive (p=0.0045). And, the "abnormal (n=141)" sign (which was not well defined in the data) was associated with a protection from being test-positive with a risk 0.65 times (OR=0.654, CI=0.44, 0.972) as likely to be confirmed as rabid (p=0.0358).

The behaviors like human aggression (n=67 & p=0.1177), wild animal aggression (n=12, p=0.6124), and Object aggression (n=25, p=0.4036) were not significantly associated with the test results.

The study carried out in Massachusetts during 1992-2006 showed the relationship between the submitter observations and final animal rabies diagnosis by logistic regression. The results suggested that aggression (OR=3.94, p<0.0001), disorientation (OR=1.17, p<0.006), paralysis (OR=1.22, p<0.041), unexplained wound (OR=1.472, p<0.0001), and found dead (OR=1.16, p=0.0089) were independently associated with positive rabies testing results (Wang, Smole et al. 2008).

The other study carried out in Massachusetts during 1992-2007 showed that the observed signs (aggression, paralysis, ataxia, disorientation, unexplained wounds) of diseases were significantly associated with rabies test results, however; seizures and animal found dead were negatively associated with the diagnosis of test results (Xingtai Wang 2010).

These studies also support our finding that certain animal behaviors could be an indicator for positive testing to some extent, which emphasizes the care people, must take while handling the suspected animals.

We also analyzed the data indicating the manner people get exposed from raccoons. We considered two manners: bite (n=4) and salivary contact (n=56) to test which of them are more commonly associated with human exposure. However, in this limited data set, there was no significant association with human exposure (p>0.05).

CONCLUSION

Raccoon rabies, in 1993 in Albany County in New York was an important public health risk. The raccoon's behaviors, age, gender, seasonality were highly associated with the positive test results. Thus these attributes of the raccoons would be helpful in planning control measures, and handling the sick or rabies suspected cases.

CHAPTER 5: OVERALL SUMMARY

Working at the Kansas State University Rabies Laboratory helped me develop my technical abilities to diagnose the brain tissues of rabies suspected animals, and to handle the large volume of samples with proficiencies. The experience obtained will, of course, be an asset, and will help me in providing inputs to the smooth running of diagnostics laboratory of Nepal.

The global presence of rabies is making the life of poor people and children in peril. This disease is at the least prioritization in the most of the developing nations of the world, particularly in Asia, where 56% of global death due to this disease occurs. The farming communities are mostly affected due to their association with domesticated and wild animals, and their poor awareness towards the disease.

In Nepal, we found that during 2000-2009, the disease is evenly present throughout the country in all seasons. The large numbers of cases were found in the areas having higher population density and having forests. Altogether 1713 animal rabies cases were recorded throughout the country. Out of 75 districts of the country, 59 districts had the evident of cases during the study period. Cattle (35.5%) and dogs (32%) were mostly affected animal species. February had the highest number of cases and May had the least. The hilly (58% of all cases) and terai region (36% of all cases) of Nepal had the maximum number of cases, which may be due to the highest population density of animals and people in these areas.

In the Albany County of New York, we found that 74.2% of raccoon brain samples were positive through Fluorescent Antibody Test. The females accounted for 57% of the positive cases indicated an association between gender and positive test results (p<0.01). As adult raccoons were mostly submitted (82%), most of them were positive (92%), while only 8% of young raccoons were positive for rabies test. We found that aggression against "domestic animals" were 4 times (OR=4.121, CI=2.150, 7.897) more likely to be tested positive rabies case (p<0.0001). The "unafraid" behavior of raccoons were 2.34 times (OR=2.34, CI=1.232, 4.447) more likely to be tested as positive rabies case (p=0.0094). The raccoons, which were "active by day time" were 1.4 (OR=1.466, CI= 1.009, 2.130) more times likely to be tested positive in the diagnosis of

brain samples (p=0.0045). The "abnormal (n=141)" sign (which was not well defined in the data) was associated with a protection from being test-positive with a risk 0.65 times (OR=0.654, CI=0.44, 0.972) as likely to be confirmed as rabid (p=0.0358).

Based on this data, the behaviors like human aggression (p=0.1177), wild animal aggressions (p=0.6124), and object aggression (p=0.4036) were not significantly associated with the test results. From these results, we can conclude that the age, sex, seasonality and observed behavior of raccoons are highly associated with the positive test results, and these factors should be taken in consideration while handling the rabies cases suspected cases in raccoons.

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