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HEPATITIS E SEROPREVALENCE STUDY IN CANIS LUPIS FAMILIARIS AND ASSOCIATIONS TO HUMAN OWNERS, SMITH COUNTY, TEXAS

Andrea Grzybowski

HEPATITIS E SEROPREVALENCE STUDY IN *CANIS LUPIS FAMILIARIS* AND ASSOCIATIONS TO HUMAN OWNERS, SMITH COUNTY, TEXAS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

HEPATITIS E SEROPREVALENCE FEASIBILITY STUDY IN *CANIS LUPIS FAMIIARIS* AND SURVEY LINKS TO HUMAN OWNERS, SMITH COUNTY, TEXAS

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Introduction: Sporadic, acute Hepatitis E is emerging more frequently in humans in the developed world, including Texas, and may be more abundant than we realize. One US human seroprevalence survey found HEV IgG rates of 21%. Many theorize a zoonotic nature of transmission; antibodies have been detected in a wide range of mammals. A recent study found 40% HEV prevalence in laboratory rabbits. Currently the USDA and CDC place suspicion on feral pigs. However, studies in Asia have found HEV in up to 23% of domestic canines – could man's best friend be a vector of transmission?

Methods: A seroprevalence study was conducted in canines from Smith County, Texas to see if HEV is present in northeast Texas and to explore if canines could be vectors due to their behavior. Canines were selected from three sites representing different care categories: a holding facility for strays/abandoned animals, a shelter for owner-surrendered canines, and a private veterinarian clinic.

Results: Specimens were drawn from 144 canines. Of 143 ELISA tests, 57 were negative, 34 indeterminate, and 52 positive. Discounting indeterminates, overall HEV prevalence was 47.7%. Site prevalence was 18.4% holding facility, 77.8% shelter, and 48.6% private clinic. Other HEV predictors were: owners' knowledge of zoonotic disease, owner's familiarity with zoonoses, and pedigree.

Conclusion: The ubiquitous nature of HEV in canines is evident by its high prevalence and distribution among sites. A model of transmission to humans remains to be found. The next step will be to test owner/pet dyads and isolate HEV RNA to determine if these pairs carried the same viral strain.

CHAPTER ONE: INTRODUCTION

Background and Significance

Hepatitis E (HEV) is considered a newly emerged infectious disease, increasingly detected in both humans and canines in countries previously considered free of risk. While shown to be fatal to both pregnant women and other immunocompromised persons (Aggarwal, Kini, Sofat, Naik, & Krawczynski, 2000; Bradley, 1992; Chandra, Taneja, Kalia, & Jameel, 2008), little is known about its transmission outside of developing countries. Previous seroprevalence studies done in China, India, and Vietnam have found 20-25% of *Canis lupus familiaris* (domestic canines) tested were positive for HEV Immunoglobin G (IgG) antibodies (Zhang *et al.,* 2008; Liu *et al.,* 2008; Arankalle *et al.*, 2001; Tien *et al.*, 1997); additionally, a study done in the United States found 21% of the humans tested were positive for HEV IgG (Kuniholm *et al.*, 2009). The potential zoonotic link between canines and humans has yet to be determined – though swine have been established as viral reservoirs (Chandra *et al.*, 2008). However, given canines' predilection for contact with decaying or fecal matter and the recent discovery of a Hepatitis C homolog in canines that is phylogenetically closer to human Hepatitis C (HCV) than any other agent (Kapoor *et al*., 2011), an investigation into the prevalence of HEV in canines as well as the potential zoonotic link between humans and canines warrants consideration.

Purpose of Study

This thesis explores whether canines in Smith County, Texas have come into contact with HEV and whether there may be any basis for further exploration into the possibility of canines being a mode of transmission to humans. By establishing a prevalence rate in canines, then by linking canine seroprevalence to canine owner information, this thesis will attempt to answer the following four questions: 1. Is there any evidence that HEV has infected canines residing in Smith County, Texas? 2. Is there any demographic parameter (age, breed, gender, location, etc.) that makes canines more or less likely to be positive for anti-HEV antibodies? 3. What do involved human owners – those who take an active interest in the health and well-being of their canines – know about zoonotic diseases in general and HEV in specific? and 4. Is there a link between involved human owner's knowledge or the canine's environment and canine positivity for anti-HEV bodies?

To accomplish these aims, blood and demographic information was collected from canines representing strayed, owner surrendered, or owned status, residing in Smith County, Texas. Laboratory analysis was performed by the BioTang laboratory (Albuquerque, New Mexico) using enzyme linked immunosorbent assays (ELISA) to determine whether any canine has developed antibodies (IgG) to HEV. Finally, questionnaires, distributed to the human owners of the clinic canines, were analyzed to determine general knowledge of zoonotic diseases of involved pet canine owners in Smith County. The following section will briefly describe previous HEV studies, biological and genotypical information, a history of outbreaks, discussions of its emergence in both developed and developing countries, and zoonotic characteristics.

CHAPTER TWO: LITERATURE REVIEW

Beginnings

First identified thirty years ago, HEV has cumulatively affected millions of people worldwide. Once thought to only affect those who lived in, or traveled to, areas of high endemicty (developing countries), HEV is increasingly found in those who live in, and have never set foot out of, areas previously thought to be free of this disease. Currently, the mode of transmission of this later, non-travel type (genotypes 3/4 – see *Biological* section below), as opposed to human (genotypes 1/2) HEV in developed countries is unknown; however, canines are a prime suspect due to their natural habits and their wide-spread appeal.

Due to the development of more sensitive bio-markers, a raging hepatitis epidemic in India, in 1980, was found to be due neither to Hepatitis A nor to Hepatitis B. It was then that scientists first described enteric non-A, non-B Hepatitis (Bradley, 1992) – which would later be renamed Hepatitis E. HEV is an infectious viral disease which presents with symptoms of acute hepatitis (Aggarwal *et al*., 2000). The disease is seldom found in children, affecting instead those ranging from 15 to 40 years of age, and is more prevalent in males than in females (Arkankalle *et al*., 1994; Arkankalle *et al*., 1995; Chandra *et al*., 2008; Hoffnagle, Nelson, & Purcell, 2012). Consequences of infection range from completely asymptomatic to jaundice, nausea, abdominal pain, fever, liver enlargement, and, in extreme cases, death (Aggarwal *et al*., 2000; Chandra *et al*., 2008).

For the general populace, HEV is rarely fatal. With a case fatality rate of less than 0.1%, most demographic groups have nothing serious to fear from the disease (Patra, Kumar, Trivedi, Puri, & Sarin, 2007). However, for women who are pregnant, the fatality rate makes a drastic spike, rising to 18-25% (Bradley, 1992; Aggarwal *et al*., 2000). One cohort study, conducted in India between 2003 and 2005, recorded a fatality rate of 41% for pregnant women who presented with jaundice and were diagnosed with HEV. In addition, those with suppressed immune systems and/or pre-existing liver conditions also had both higher chronic infection as well as fatality rates (Chandra *et al*., 2008; Labrique, Kuniholm, & Nelson, 2010).

Biological and Genotype Information

The virus responsible for HEV was isolated and identified in 1983 using immuno electron microscopy. It was also at this time that the ability of the disease to be transmitted to humans was first confirmed (Arkankalle *et al*., 1994). In the early 1990s, Reyes *et al.* were able to clone and sequence part of the HEV genome and develop an enzyme linked immunosorbent assay (ELISA) to test for HEV antibodies (Arankalle *et al*., 1994; Reyes *et al*., 1990). Later that year, Tsarev *et al.* (1993) were able to create an ELISA that specifically picked up both HEV Immunoglobin M (IgM) and IgG.

The virus that would eventually become known as hepatitis E genotype 1 was first identified in 1990 (Reyes *et al*., 1990). Between 1990 and 2005, three additional genotypes were identified and characterized (Heseltine, 2012). These four genotypes/strains are represented by the geographic area (Figure 1) in which they were first isolated (Lu, Li, & Hagedorn, 2006). Genotypes 1 and 2, Burmese and Mexican, respectively, are limited to primate hosts. These genotypes are typically responsible for

large scale epidemics in developing countries as well as travel-related HEV (Heseltine, 2012) and generally considered to be non-zoonotic. Genotypes 3 and 4, respectively US and Chinese isolates, are zoonotic strains found in both developed and developing countries. Typically, those who contract sporadic non-travel-related HEV contract genotype 3 or 4.

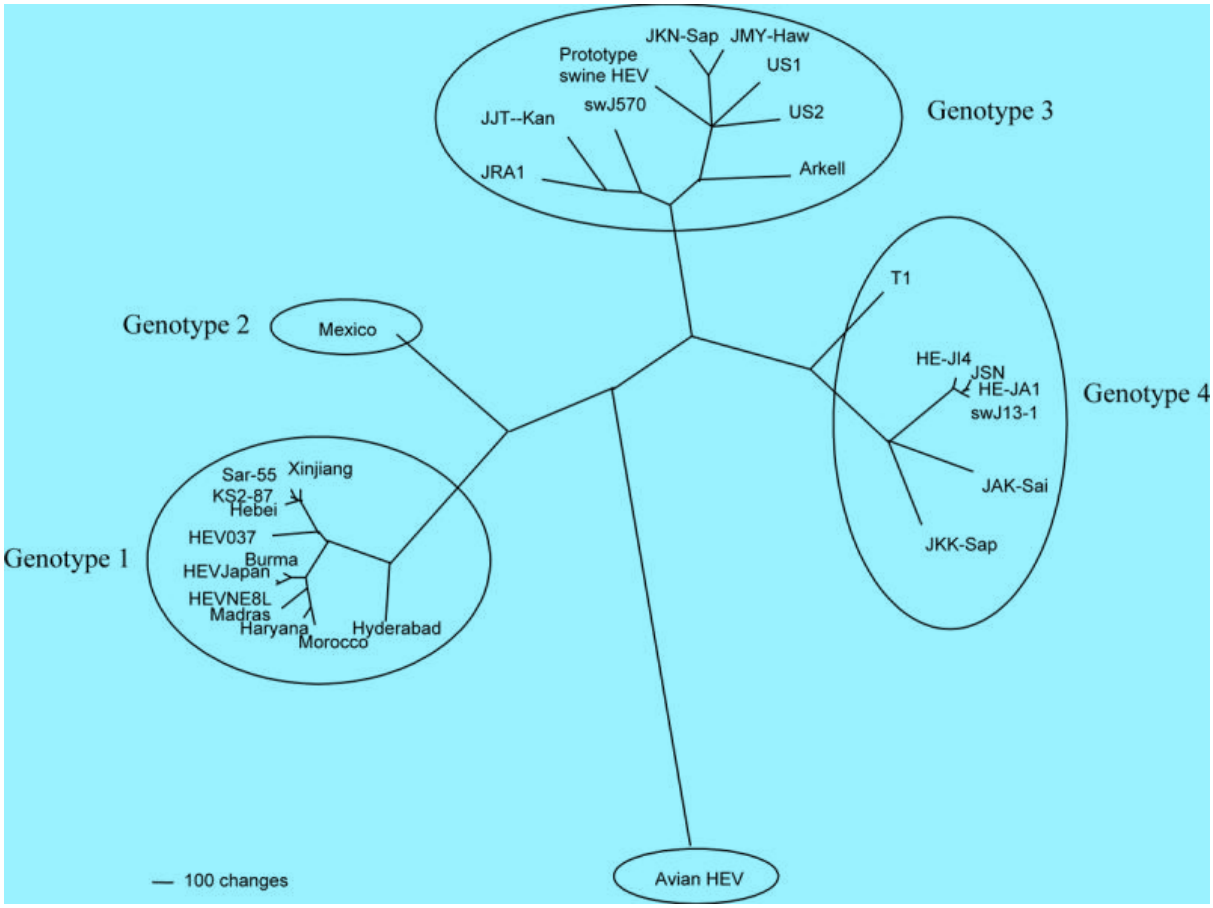

FIGURE 1. Unrooted HEV Phylogeny (Meng, 2010)

Initially classified in the *Caliciviridae* family, HEV was reclassified in the new *Hepeviridae* family as the sole member of the genus *Hepevirus*, in 1988, by the International Committee for Taxonomy of Viruses (Chandra *et al*., 2008; Meng, 2000; Montalvo *et al*., 2010). After the disease was identified, retrospective studies on previous

acute hepatitis epidemics were conducted. Using serum samples which had been preserved, a number of large-scale epidemics of enterically transmitted non-A, non-B Hepatitis were found to be caused by HEV (Bradley, 1992; Thomas *et al*., 1997).

HEV in Developing Countries

The first retrospectively documented occurrence of epidemic HEV was the 1955, New Delhi, India outbreak. With almost 30,000 reported cases, this outbreak had been attributed to Hepatitis A until the discovery of HEV. Between 1955 and 1956 another outbreak, inducing 10,800 cases, struck in modern day Kyrgyzstan, resulting in an 18% case fatality rate in pregnant women. However, HEV would strike again, between 1975 and 1978 when over 20,000 cases were reported from India and Burma (Bradley, 1992). HEV's latest outbreaks began in Sudan in July of 2012 and continue today – with over 6,000 cases and over 140 human deaths (including a 10.4% case fatality in pregnant women [Hepatitis E – South Sudan, 2013; Hepatitis E – Sudan, 2014]). With over two billion people living in endemic areas (Chandra *et al*., 2008), this disease continues to make its presence known on the world stage.

As recently as 2000, HEV was rarely found outside of subtropical-tropical developing countries where it is the principal cause of acute Hepatitis (Aggarwal *et al*., 2000; Meng *et al*., 1998). Spread through the fecal-oral route, often through contaminated water, HEV genotypes 1/2 are the main source for explosive, large scale epidemics (Meng *et al*., 2002). However, over the last 12 years cases of HEV have begun to crop up in developed countries across the globe.

HEV in Developed Countries

Beginning in the 1980s, sporadic human cases of HEV genotypes 3/4 began emerging across the globe. For example, during the 1980s, 13 cases of HEV were reported in the United Kingdom. The patients had no contact with swine or anyone who worked with swine and reported only eating fully cooked pig products. It was not until the 1990s that these samples were sequenced and genotype 3 was discovered to be the culprit (DeSilva *et al*., 2007). In recent years, both the number of cases and frequency of outbreaks have increased. For instance, in Japan, five members of a family and two of their close friends all contracted genotypes 3/4 HEV in 2003. It was found to be caused by the consumption of raw deer meat that, when sampled and tested, had the identical HEV RNA strain as that of the patients (Shuchin, Naoto, Kazuaki, & Shunji, 2003). In 2004, a case with genotypes 3/4 HEV was reported in El Paso, Texas; again, the claim was made that the patient had no contact with swine (Amon *et al*., 2006).

Over the course of the next two years in the Netherlands (2004-2006), 13 more people contracted the disease. While none of these patients had any contact with swine, 74% of them reported having at least sporadic contact with canines – of those, 32% owned their own canine (Borgen *et al*., 2008). Germany was another site whereby a non-traveler, this time in 2008, contracted HEV genotypes 3/4 (Tessé *et al*., 2012). This was followed in 2009 by two cases in San Antonio, Texas. Neither of the patients in San Antonio reported contact with pigs or uncooked pig products. While one was a heavy drinker and drug user, the other, who died one month after hospitalization, was reported as owning five canines and one feline (Tohme *et al*., 2009). In the last three years, more cases of genotypes 3/4 HEV have been reported: five cases in Italy and another case in

France, all between 2011 and 2012 (Garbuglia, 2013; Tessé *et al*., 2012). The single case in France mushroomed into an outbreak with over 280 people being confirmed as having contracted genotype 4 HEV. This outbreak, in part, was linked to the patients consuming figatelli – an uncooked liver sausage (Jeblaoui, Haim-Boukobza, Marchadier, Mokhtari, & Roque-Afonso, 2013). A common factor in most of these cases, excluding the Japanese and French outbreaks, is that no one has been able to determine the source of infection.

With the advent of the anti-HEV IgM and IgG ELISA techniques in the 1990s, the increasing occurrences of HEV genotypes 3/4 infections, and the fact that infection can be asymptomatic, researchers began conducting large scale anti-HEV IgG seroprevalence assays. While IgM is only in the body briefly – no more than 4-5 months after the acute phase of the disease – IgG can be detected 1.5-4 years after the acute phase, and even as long as 14 years later (Aggarwal *et al*., 2000; Khuroo, Kamili, Dar, Moecklii, & Jameel, 1993).

In 2009, Kuniholm *et al.* released their findings from a study conducted on blood serum samples that were part of the National Health and Nutrition Exam Survey III. This survey spanned from 1988 to 1994 and involved over 18,000 individuals from diverse socioeconomic backgrounds from across the US. It was found that 21% of the population had antibodies to HEV. Results indicated, having a pet, or more specifically a canine, made one 1.2 times more likely to test positive than if one did not own a canine (Kuniholm *et al*., 2009).

In 1997, results from a study of both high risk adults and blood donors in Baltimore, Maryland were released. Using blood samples and data collected from 300 homosexual males, 300 injection drug users, and 300 blood donors as part of a larger

HIV study program, it was found that all three groups yielded positive HEV antibodies with 16%, 23%, and 21% prevalence respectively (Thomas *et al*., 1997).

Another study conducted in the US in 2002, this time on swine veterinarians and workers as well as blood donors, found that 23% of veterinarians and swine workers tested positive regardless of how much time they spent working with swine. Conversely, 18% of blood donors from the same states as the veterinarians and workers (Minnesota, Indiana, Nebraska, Iowa, Illinois, Missouri, North Carolina, and Alabama) tested positive as well (Meng *et al*., 2002).

In Germany, between 2008 and 2011, researchers collected over 4,300 serum samples from native adults from across the country. They found that 16.8% tested positive for anti-HEV bodies and none of them reported either traveling to endemic countries within the three months prior to the test, coming into contact with swine, or eating uncooked swine products (Faber *et al*., 2012). In Cuba in 2009, blood was drawn from over 450 healthy individuals who reported never having jaundice and had no history of viral hepatitis; 10% tested positive for HEV IgG (Montalvo *et al*., 2010). With the increase of individuals in developed countries positive for IgG and the discovery that HEV could be transmitted across species, the hypothesis has arisen that, in cases of HEV genotypes 3/4, there must be a zoonotic link (Meng *et al*., 2000; Meng *et al*., 2002). While the route(s) of transmission still remain a mystery, possible suspects have arisen.

HEV's Zoonotic Background

HEV genotypes 3/4's ability to cross species (from swine to humans) has prompted extensive studies of HEV in swine. Across the globe, HEV has been studied at every age of domestic swine and found that the highest levels of HEV RNA are found in

those pigs less than two months old. As many as 60% of pigs in their first few months of feeding have been found to have HEV RNA in their feces (Fernandez-Barredo *et al*., 2006). While tests on swine have been extensive, tests on household pets have been far less so.

The few studies conducted were in China, India, and Vietnam and did not include much background on the animals. The biggest animal study was from Shanghai where cows, goats, horses, ducks, pigeons, chickens and canines were all tested for anti-HEV bodies. The antibodies were found in all species. The highest rates were in goats (24%) and canines (18%); however, the sample sizes were small-with only 101 canines being tested (Zhang *et al*., 2008). Another study conducted in China looked at almost 200 pet canines and tested their blood for HEV IgG. Of the 192 tested using ELISA, 26 (14%) tested positive (including the two canines they injected with swine HEV). However, when reverse transcriptase polymerase chain reactions (RT-PCR) were run, no HEV RNA was found in any of the fecal samples (Liu *et al*., 2009). Similarly in India, where 23% of the canines tested were positive for HEV IgG, no RNA was able to be isolated from fecal samples (Arankalle *et al*., 2001). The largest seroprevalence in canines identified 27% positive (Vietnam), but little demographic information was reported on the canines tested (Tien *et al*., 1997). Conversely, a study conducted in Japan found a 0% prevalence rate in canines – though this may be due to any number of extenuating factors including small sample size (Mochizuki *et al*., 2006). All of these studies had small to modest sample sizes of canines with very little demographic background information gathered on the canines themselves.

More recent studies into infected human food products have turned up surprising results. A study conducted in the US found that 10% of samples of commercially available swine livers were contaminated with HEV and that those viruses were still infectious at point of sale (Feagins, Oprissnig, Guenette, Halbur, & Meng, 2007). Another study conducted in slaughterhouses, processing plants, and points of sale in the UK found HEV in all areas tested (Berto, Martelli, Grierson, & Banks, 2012). Furthermore, in Europe, Italy had the highest contamination rate (53%) in a multi-country study of slaughterhouses and points of sale. Several of the strains identified in Spain were genotype 3 (Di Bartolo *et al.*, 2012). This, coupled with a study that found one out of four contaminated pork food stuffs were still capable of HEV replication (Berto *et al.*, 2013), demonstrates a clear need for answers.

In the thirty years since HEV was first identified, swine have been established as a reservoir of genotypes 3/4 HEV (Chandra *et al*., 2008); however, since the mode(s) of transmission still remains a mystery – as many people in metropolitan areas and those with no contact with swine are positive for IgG antibodies, it appears that pets may be accidental hosts (Kuniholm *et al*., 2009). Given canines' predilection for contact with decaying or fecal matter and their human owner's responses to those behaviors (bathing them, cleaning up after them, etc.), it is possible that canines could be a key mode of transmission to humans from a larger reservoir.

CHAPTER THREE: METHODS

Subjects

The original sampling plan for this seroprevalence study consisted of testing 105 canines, 35 chosen at random from each of three different locations: an animal shelter, a stray canine holding facility, and a veterinary office. The sample for the questionnaire consisted of the owners of the 35 canines from the veterinary clinic. All canines and owners came from Smith County, Texas, with a majority centered in the city of Tyler, Texas. Smith County, located in northeast Texas, is a semi-urban, wooded area home to more than 210,000 people (Census Bureau, 2013) who own approximately 48,252 canines (American Veterinary Medical Association, 2013).

The stray canines holding facility (HF) is the Smith County Animal Control holding facility. This facility is where nuisance canines located in rural Smith County (i.e. outside of the city of Tyler) are brought when picked up by either the Smith County Sheriff's Office or Smith County Animal Control. These canines are held for three days and then humanely euthanized if not claimed by owners or rescue groups within that time period. The facility itself is located in the city of Winona, TX. These canines most likely had little to no human owner involvement as well as being those most likely to roam more rural areas.

The second group of canines sampled were from the Pets Fur People (PFP) (formerly Humane Society of East Texas) shelter – a no-kill, selective admissions facility

where those owners who surrender canines are required to pay a fee in order to do so. This shelter is located in Tyler, TX. This site represents those canines with medium owner involvement – the owner gave them up (or a Good Samaritan found them and brought them in), but they did so in a responsible manner – by transporting them, and paying to leave them at a shelter. This implies that some kind of financial investment had been made in the canine at some point. It is thought that these canines will be less likely to roam than the strayed canines, but more likely to have been free-roaming than the last group of canines.

The third, and last, group of canines came from the Tyler Veterinary Center (TVC) in Tyler, TX. These canines and their owners were recruited during routine office visits – the canines for blood draws and the human owners for participation in a questionnaire. These canines represent high owner involvement – all canines seen there were brought in by an owner for a full complement of vaccinations (rabies, parvo/distemper, bordatella, etc.) as well as general checkups or emergency care.

Inclusion criteria for all canines included: age of at least two years (to ensure that immune system is fully formed); a healthy appearance (not disabled, for example); and not displaying aggressive behaviors. All final decisions regarding suitability of individual canines as study subjects was determined by Sharon Phillips, DVM, who also conducted all venipunctures and blood collection between the months of November and December 2013. Inclusion criteria for humans were: over 18 years of age, owner of canine brought into the clinic, and willingness to participate.

Procedures

Institutional Review Board and Institutional Animal Use and Care approval (Appendix A) was granted through The University of Texas at Tyler (UT Tyler). The Smith County holding facility, Pets fur People, and Tyler Veterinary Clinic were approached for approval to use their animals for this study, and to arrange collection dates. Agreements were documented (Appendix B). The collection dates for the holding facility were the days prior to euthanizing as this was the day when their barn was fullest. The collection date for the shelter was determined by veterinarian availability; that facility is consistently occupied. The collection dates for the clinic were over the course of a week to allow for as many canines to come in as possible. All staff members were given an informational flyer on Hepatitis E to reduce the spread of the disease and alleviate any apprehensions regarding potential exposures (Appendix C). All site managers were informed that their facility would not be cast in a negative light and that only the conglomerate results of the seroprevalence study would be made available to the public, not the location, conditions, or business practices of the individual facility. All staff members were informed that they were free to ask questions about the study design or HEV at any time. Individual test results were not returned to holding facility or shelter staff because, respectively: 1. Holding facility canines were euthanized or moved to rescue groups by the time results were returned and 2. Shelter canines may have experienced undue prejudice including, but not limited to, humane euthanasia due to a positive test result. Since this infection has no cure, but also at this time no known zoonotic link, no beneficial outcome is foreseen in reporting individual outcomes.

Surveys

Survey participants were recruited via convenience sampling. All human participants were informed that their answers would be kept anonymous and confidential, that they were free to ask questions of the investigator regarding study design at any time, that they were free to skip any question(s) they felt uncomfortable answering, and that they were eligible to receive the results of their canine's screening if they so chose. Owners were also given the same educational flyer as staff following administration of the survey.

Once the owner verbally consented to participate, they were given the paper survey while their canine had its blood drawn. Survey questions (Appendix D) were formulated using Fowler's recommendations on question design (2009). Survey questions consisted of demographic and behavioral inquiries about the owner's canine. All surveys were administered in exam rooms and collected prior to the owner leaving. All participants were asked to complete the survey on their own without the use of the internet or others in order to increase the accuracy of the survey information. Multiple choice, yes/no, Likert scale and fill in the blank questions were utilized. The surveys were content-validated by two professors with experience in survey design. The investigator or the veterinarian was present during the survey administration to answer questions regarding the content of the survey. Participants were not compensated for participation in this study, nor were outside investigators.

Laboratory Analysis

When a human or canine contracts HEV, IgG does not appear until several weeks after the acute phase, and can be detected 1.5 to 4 (and as high as 17) years after initial

infection (Aggarwal *et al*., 2000; Khuroo *et al*., 1993). Thus all blood specimens were tested using ELISA for anti-HEV anti-body. Genotyping by Rt-PCR will follow any positive samples as determined by Dr. Ali Azghani (Biology, UT Tyler). Funding sources for PCR testing at this time are unknown; however, there is a possibility of collaborating with the USDA for future testing.

Transport, storage, and analysis of blood specimens were done according to the manufacturer recommendation for the indirect ELISA analysis (BioTang, Albuquerque, NM.). Three to five ml of blood were collected in red top Vacutainer® tubes from either the neck or forepaw of the canine (Appendix E). Serum was separated as soon as possible to avoid hemolysis. Aliquots of samples were stored at -20 ºC until use. Specimens were microfuged immediately prior to assay to avoid false positive results and ran in duplicates. Optical density (OD) of the reactions in the ELISA plates was read at 450 nm using a microplate reader (Beckman Coulter AD 340). The average of duplicate readings for samples and controls (positive and negative) were used. The positive/negative (P/N) values were determined by: $P/N = OD_{sample}/(OD_{positive} \text{ X } 10\% + OD_{negative})$. Samples with $P/N>1.0$ were considered positive while those with $P/N<1.0$ were called HEV IgG negative. All ELISA analysis was supervised by faculty from the Biology Department at UT Tyler (Azghani, Baker, Shetty, Miller, & Bhat, 2002). The ELISA component (disposable laboratory equipment) was funded by the Department of Health and Kinesiology at UT Tyler.

Data and Analysis

Blood samples were drawn by a licensed veterinarian from 105 (sample needed for 95% confidence and standard statistical power) canines. All canines that met

inclusion criteria were tested at three collection locations. One may describe selection as quasi-random for any given day canines randomly entered and left a facility, but at the day of specimen collection canines were conveniently selected. For those canines recruited from TVC, the "owner" is the adult individual who transports the canine to the clinic. If a couple transported their canine at the same time, the human male was recruited. Recruited humans received full disclosure and informed consent.

Demographic information for all canines included: approximate age, breed, gender, and, if available, where they were found/were from. This information was entered into an Excel spreadsheet and matched to the laboratory result.

After collection procedures were completed, all data was uploaded into statistical analysis software (SPSS v.20). Statistical analysis utilizing chi-square as well as independent t tests were run to capture bivariate significant risk groups identified by owner knowledge/beliefs, canine behaviors, and HEV positivity. Backwards logistical regression was performed from bivariate significance findings.

Post Proposal Changes

Several changes were made to the methods after the initial proposal was presented. Due to a discount from the manufacturer, 144 canines instead of the original 105 animals were tested. As a result of a lower number of animals being available at all of the locations, the lower limit on age was reduced to 1 year – this decision was made in consultation with the veterinarian. Furthermore, all animals that met the inclusion criteria were tested, not randomly sampled. Additionally, due to the high positive prevalence found in our lab and to ensure accuracy, an aliquot from each specimen was run off-site at the BioTang laboratories in Albuquerque, New Mexico.

Furthermore, in order to analyze the data, those canines indicated as being of mixed breed were moved to their over-arching breed group (e.g. a German Shepherd mix would be categorized as a German Shepherd for data analysis purposes). For those canines indicated as designer breeds (intentional cross breeding to create a mixed breed animal of only desired breeds), they were grouped with their predominant breed group (e.g. a Labradoodle was listed as a Labrador Retriever). Moreover, in order to make the number of canine breeds more manageable, the researcher used breed categories from the American Kennel Club to group each individual animal as either being historically bred for contact with animals and those that were not. Lastly, in order to adjust the knowledge section of the survey to account for participants guessing, the following weight system was used: for correct answers, participants were awarded one point; if the question was left blank, no points were gained and no points were lost; however, if the question was answered, but the response was incorrect, the participant was marked down one half point. Additionally, for those questions with multiple answers, the participant had to get all parts of the question correct in order to register knowledge for that question. These adjustments formed the knowledge score described in the results section.

CHAPTER FOUR: RESULTS

Canine Characteristics

In the seroprevalence portion of this study, 144 canines were sampled, split evenly between males and females. These canines had an average age of 4.3 years with the youngest at one year old and the oldest 14 years old (with a standard deviation of 3.3). These 144 canines represent 40 different breeds and 7 of the breed groups as defined by the American Kennel Club (2014). The samples were collected from three different locations: Smith County holding facility (HF) (n=51, 35%), PFP (n=45, 31%), and TVC (n=47, 33%). In addition, 64 (44%) canines in the sample were, or judged to be purebred (Table 1).

Survey Results

The survey administered to the human owners of those dogs recruited from TVC resulted in 36 of the 47 (77%) surveys being completed in their entirety. The average age of the TVC dogs was 6.8 years with a range of 1 to 14 years (standard deviation of 3.9 years). The quantified responses to whether each canine had any contact with either domestic or wild animals revealed that the majority of the animals from the veterinary clinic had little to no contact with any animals outside of other canines (Table 2). Furthermore, most of the animals were reported as only being outside in some form of yard for part of the day, and mostly in their own, fenced, back yard (Table 3). Those animals reported as being groomed were only rarely or occasionally groomed, and

groomed either by someone within the animal's household or done by a professional (Table 4). Finally, Table 5 shows responses to a number of yes/no questions. While some questions (fed raw meat and whether canine is a working dog) returned a mere handful of indications, others (whether animal was given chew toys or whether the owner allowed the canine to lick them) resulted in far more affirmative responses (Table 5).

The next section of the survey covered the familiarity of the participants(n=47) over zoonoses as well as their beliefs and knowledge (Appendix D). When asked how familiar the participant was with zoonotic diseases, the average response (on a scale of 1 not familiar at all, to 10 very familiar) was 2.7. When asked to rate the importance of canine vaccinations, again on a scale of 1 to 10, the average response was 9.2. Finally when asked to categorize their concern about contracting a zoonotic disease themselves, the average ranking was a 5.5.

For the knowledge section, 36 participants completed some portion it. The average score, when adjusted to account for guessing and non-responses, was 1.3 with a range of -3 to 4.5 (out of a possible 6). For the first question on the survey (Appendix D - *a zoonotic disease is*?), 12 of the 36 (33%) answered correctly with 14 (39%) not answering at all. The second question, regarding which disease is not a zoonotic disease, showed 11 of the 36 individuals (31%) answering correctly with, again, 14 non-responses. When asked how one may contract a zoonotic disease, 36% (13 of 36) answered correctly. The most difficult question for survey participants was the fourth question – *Which are not currently recognized forms of Hepatitis?* While a number of participants answered the question partially correctly, only those who indicated all three of the correct answers were marked as correct. This left only two participants answering

the question correctly. The last two questions on the survey proved to be the easiest for participants with 72% (26 individuals) answering question 5 (*what Hepatitis in general affects*) correctly, and 86% answered question 6 (*how best to prevent the spread of infectious diseases*) correctly.

Survey Results Compared with Canine Characteristics

To test for association between canine characteristics and human owner knowledge scores, the adjusted scores of the 36 who answered some part of the knowledge section were analyzed. For example, it was found that with regards to the knowledge score, gender and age of the animal affected the overall score. Although not significant, a curious trend was found: For the owners of female canines, the older the animal, the lower the knowledge score. However, the reverse was found to be true for males; for them, the older the animal the greater the knowledge score ($p=0.560$, $r=0.104$) (Figure 2).

When the knowledge scores of the owners were compared with specific behaviors of the animals (Table 6), it was found that there was significant difference between those who took their animals for walks and those who did not, with those who did not having more knowledge ($p=0.02$) (Figure 3). Owning other canines was a marginally significant indicator of knowledge with those who owned more than one canine showing more knowledge than those who did not ($p=0.093$) (Figure 4). And while the variable *canine having contact with wild animals* did not produce a significant difference in knowledge scores ($p=0.145$), it did show the mean scores moving in opposite directions from each other (Figure 5). However, no other significant association was found with regard to knowledge score and canine behavior (Table 6).

Comparing owner familiarity and beliefs with specific behaviors likewise resulted in several significant associations. Those indicating more familiarity with zoonoses were less likely to allow their canine to have contact with wild animals (p=0.009) than those with lower familiarity. Similarly, those who indicated higher familiarity with zoonoses were also significantly less likely to allow their animal to lick them (P=0.006). All other associations between familiarity and behaviors were either not significant or only marginally so (Table 6). The levels of concern over contracting a zoonotic disease resulted in only one significant association– those professing higher levels of concern were less likely to allow their canine to have contact with wild animals (p=0.000). Lastly, vaccine importance compared with specific behaviors resulted in no significant association (Table 6).

When specific behaviors of canines were analyzed against the canine's age, a similar number of significant comparisons were found. Those who owned more than one canine tended to have younger animals than those who only owned one (p=0.01) (Table 6 and Figure 6). Moreover, those canines who had contact with any form of wild animal as defined by the survey were more likely to be younger than those who did not (p=0.01) (Figure 7). And while only marginally significant, those canines who had contact with other canines outside of their household were younger than those who did not (p=0.067) (Figure 8).

Hepatitis Results

Of the 144 samples drawn, 143 were returned from the laboratory. Of these, 57 (40%) were negative, 34 (24%) were determined to be unsatisfactory for analysis due to hemolysis of the specimen, and 52 (36%) were positive. Of the 52 specimens from the

HF, 31 (60%) were negative, 14 (27%) were undeterminable, and 7 (13% were positive). At PFP, 8 (18%) were negative, 8 (18%) were undetermined, and 28 (64%) were positive. Finally, from TVC, 18 (38%) were negative, 12 (26%) were undetermined, and 17 (36%) were positive.

Due to the inability to accurately determine the OD (defined previously in the methods section) of the hemolyzed specimens, they were discounted from further analysis. In considering a denominator of 109 specimens (those that were determined), the positive prevalence rate for Smith County was 48%. Of those, backwards logistical regression demonstrated a significant difference between the facilities (p=0.00): 18% of the canines tested at the HF, 78% of those from PFP, and 49% of those from TVC were positive.

Of those canines that were positive, the majority were male (n=30, 58%) and the average age was 4.6 years. Of those that were negative, the majority were female (n=35, 61%) and the average age was 4.2. The breeds with the highest number of positivity were Labrador retrievers with 13, Chihuahuas with 7, and Beagles with 5 (Table 1). Percentages within breeds showed that 25% of all Labrador retrievers, 58% of Chihuahuas, and 60% of all Beagles tested were positive. Those animals reported as, or determined to be, of mixed breed were marginally less likely to be positive than those that were purebred (p=0.073).

Human-Canine HEV Connection

The seroprevalence data from those canines from TVC were analyzed against the survey responses (Table 6). A significant difference existed between the knowledge scores of the owners of positive canines – with those owning positive animals being more

likely to have a higher knowledge score (p=0.028) (Figure 9). Furthermore, when the owners' familiarity level was analyzed against seroprevalence, those who indicated that they were familiar with zoonotic diseases were more likely to have a positive canine (p=0.026) than those who indicated they were less familiar (Figure 10). No other significant association was found.

In order to control for each of the significant bivariate results (purebred or mixed breed, owner knowledge, owner familiarity with zoonotic diseases, and canine location), multivariate logistic regressions were performed. When implemented for those animals from TVC, it was found that familiarity with zoonotic diseases was the only predictor of canine HEV infection (p=.044), controlling for mixed breed and knowledge (Table 7). Surprisingly, for those owners expressing the highest levels of familiarity with zoonotic diseases, their animals were 67% more likely to be positive.

When the logistic regression analysis was performed on all animals two variables were controlling for each other: mixed breed and location. Both were significant predictors. Canines from PFP were 21 times more likely to be positive than those from HF (p=0.00), and those from TVC were 5.2 times more likely to be positive (p=0.005) (Table 8). Location seems to be the strongest predictor of HEV positivity.

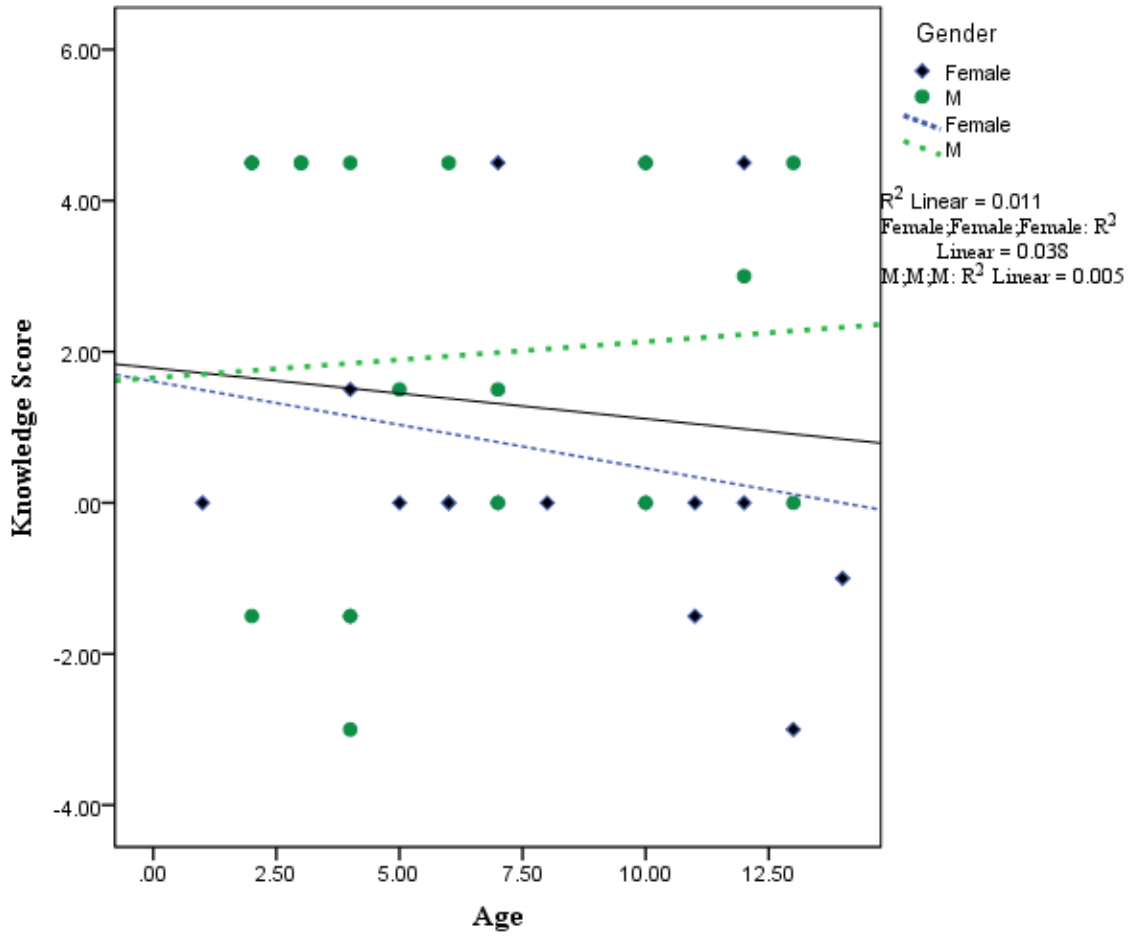

FIGURE 2. Age of Canine and Knowledge Score by Gender

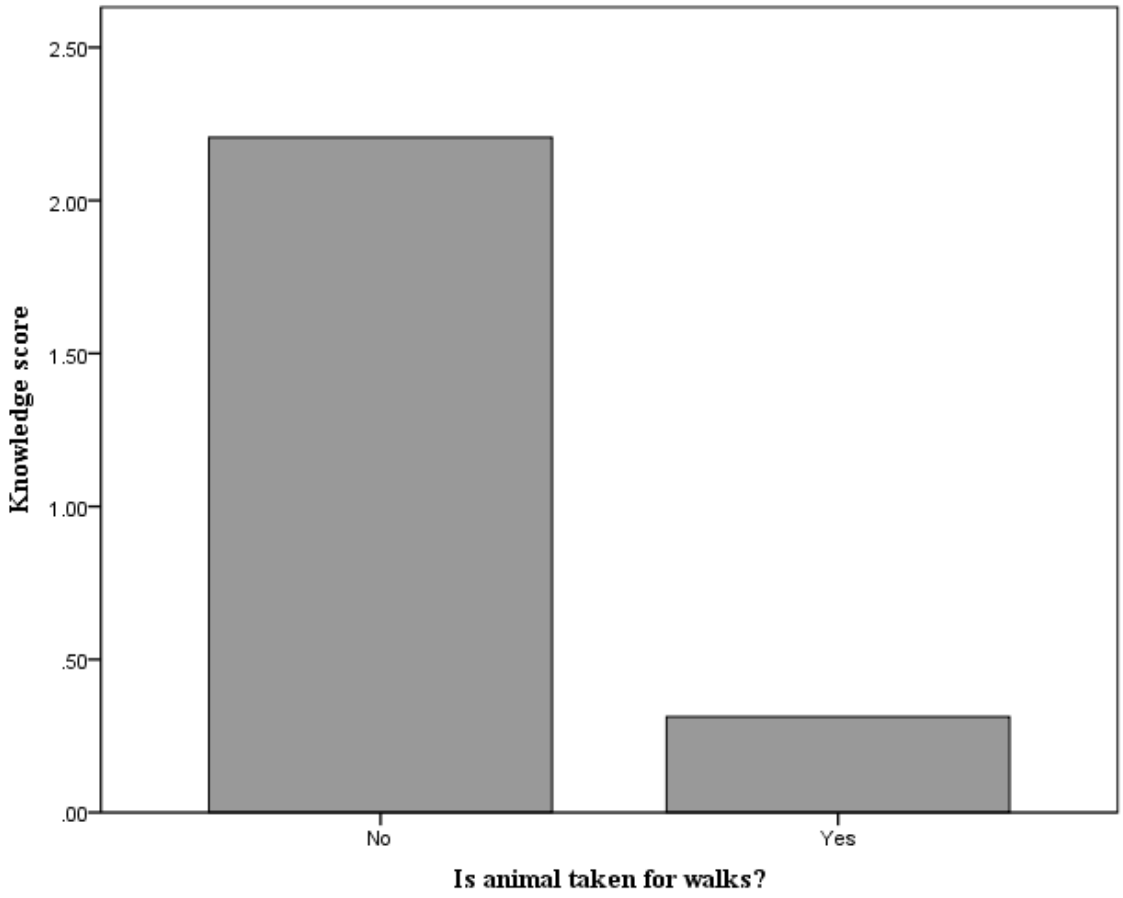

FIGURE 3. Knowledge Score by Whether Dog is Walked by Owner

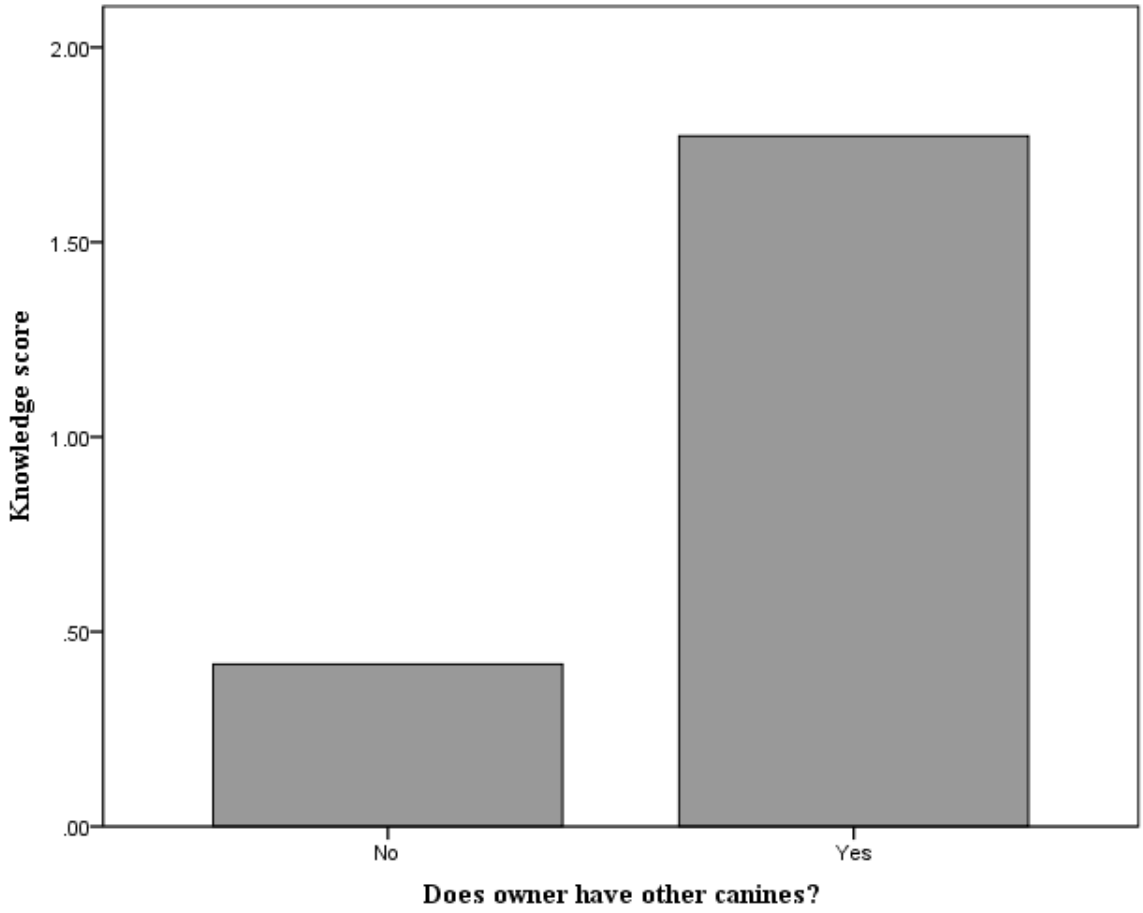

FIGURE 4. Knowledge Score by Multiple Canine Ownership Status.

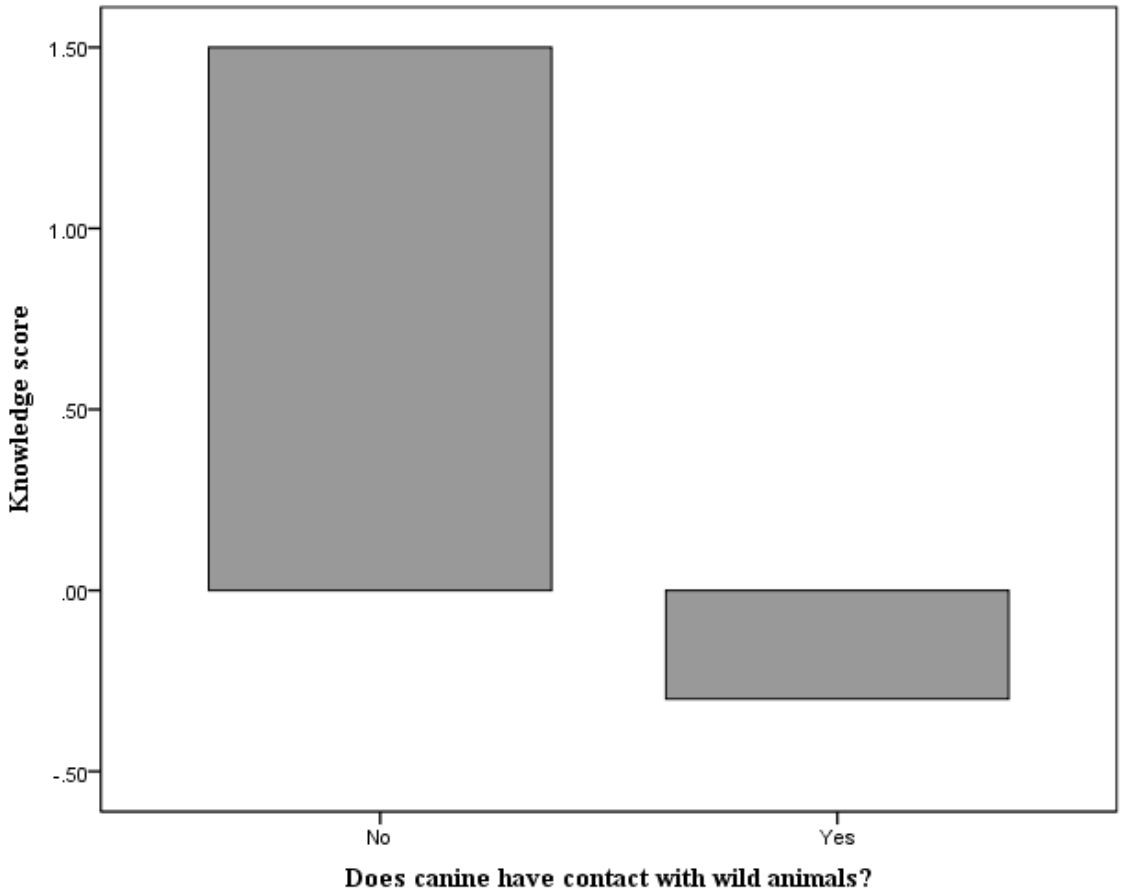

FIGURE 5. Knowledge Score by Whether Canine Has Contact with Wild Animals.

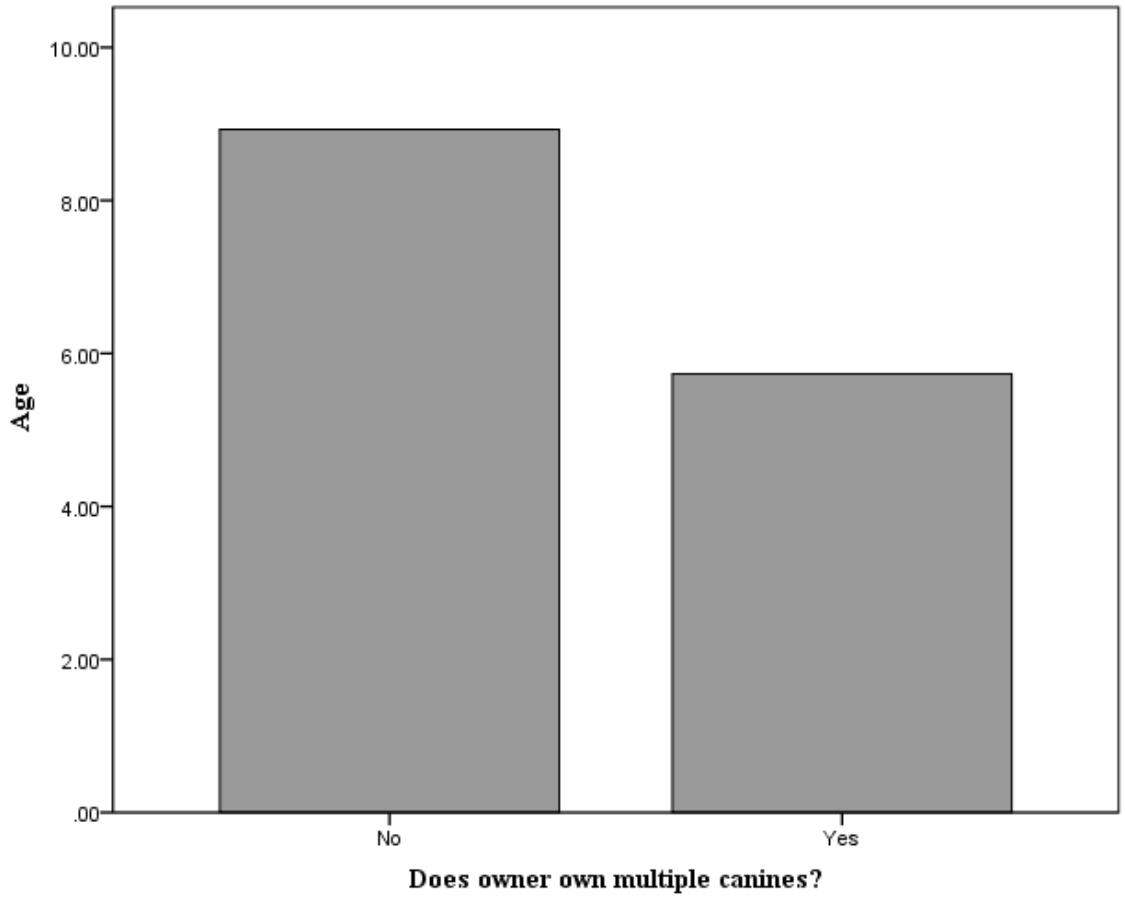

FIGURE 6. Mean Age of Canine by Multiple Canine Ownership Status.

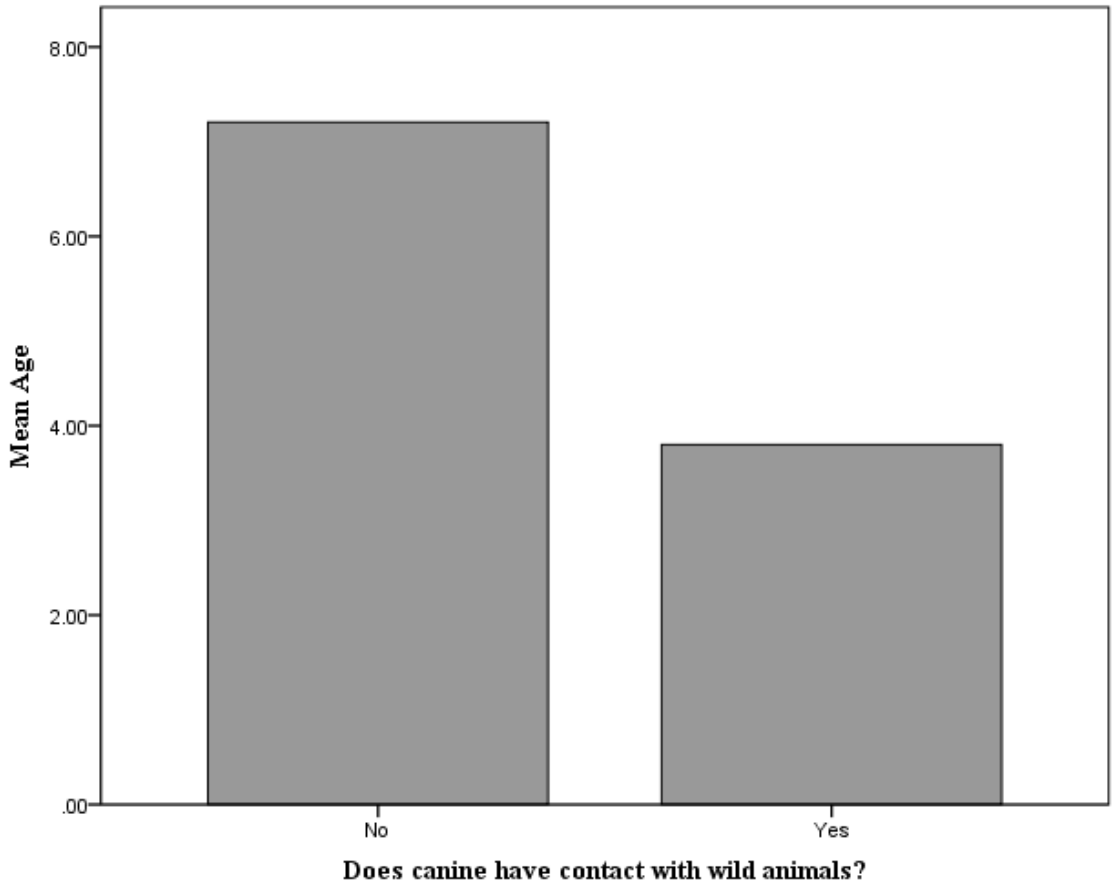

FIGURE 7. Mean Age of Canine by Contact with Wild Animals

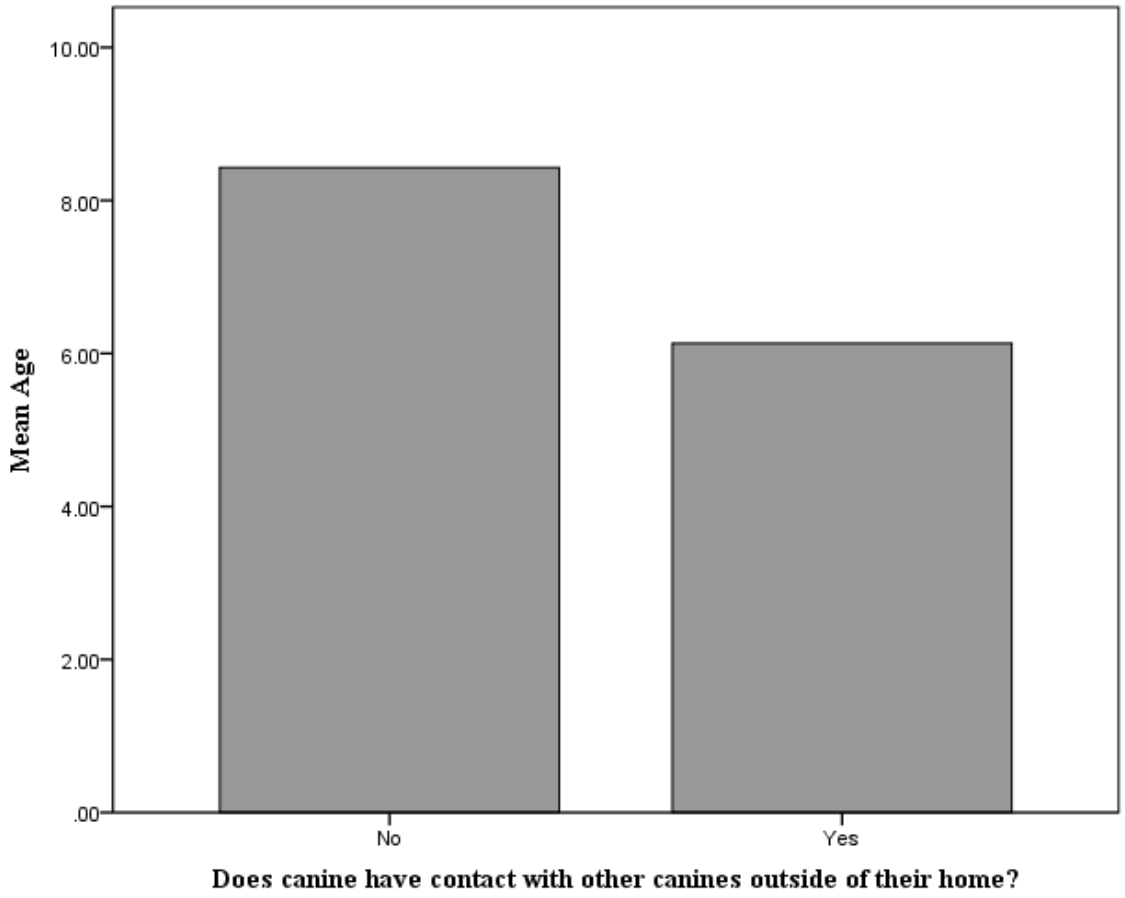

FIGURE 8. Mean age of Canine by Whether Canine Has Contact with Canines Outside of Their Home.

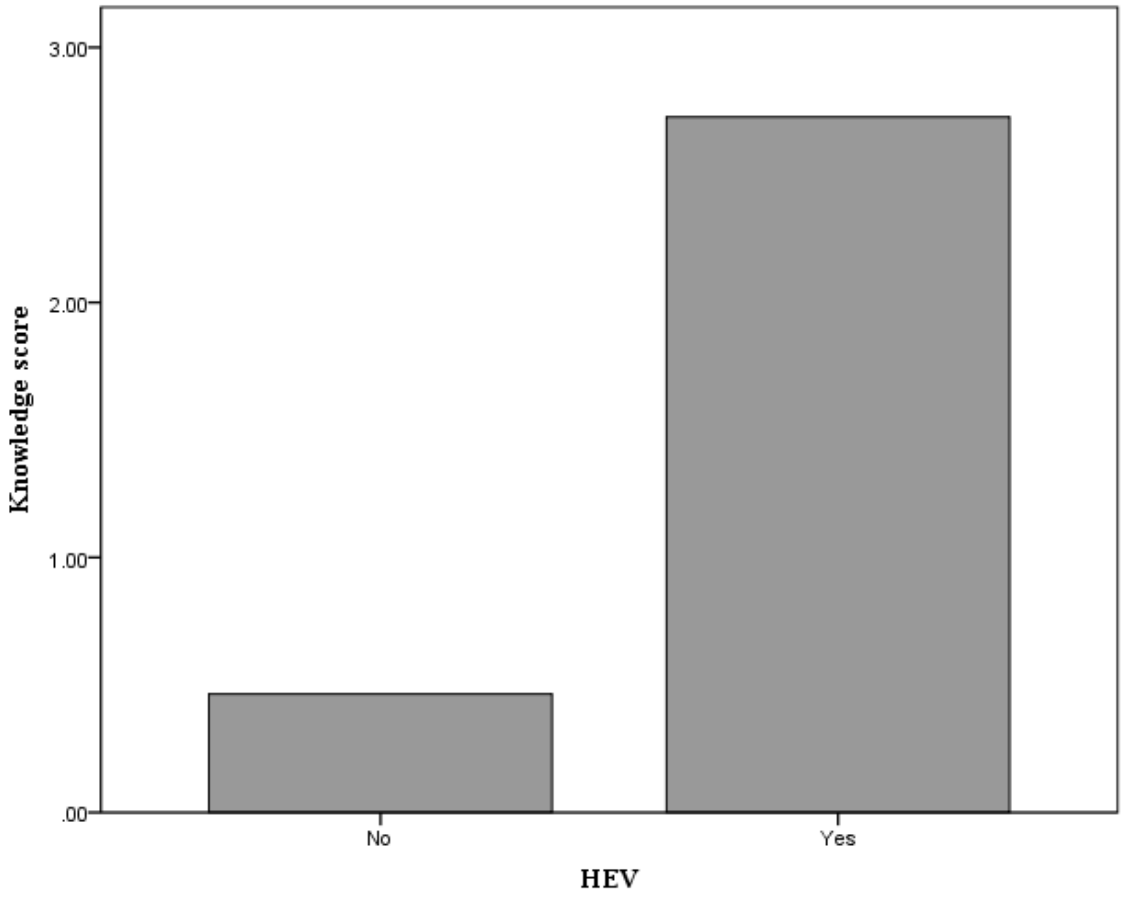

FIGURE 9. HEV Positivity by Knowledge Score

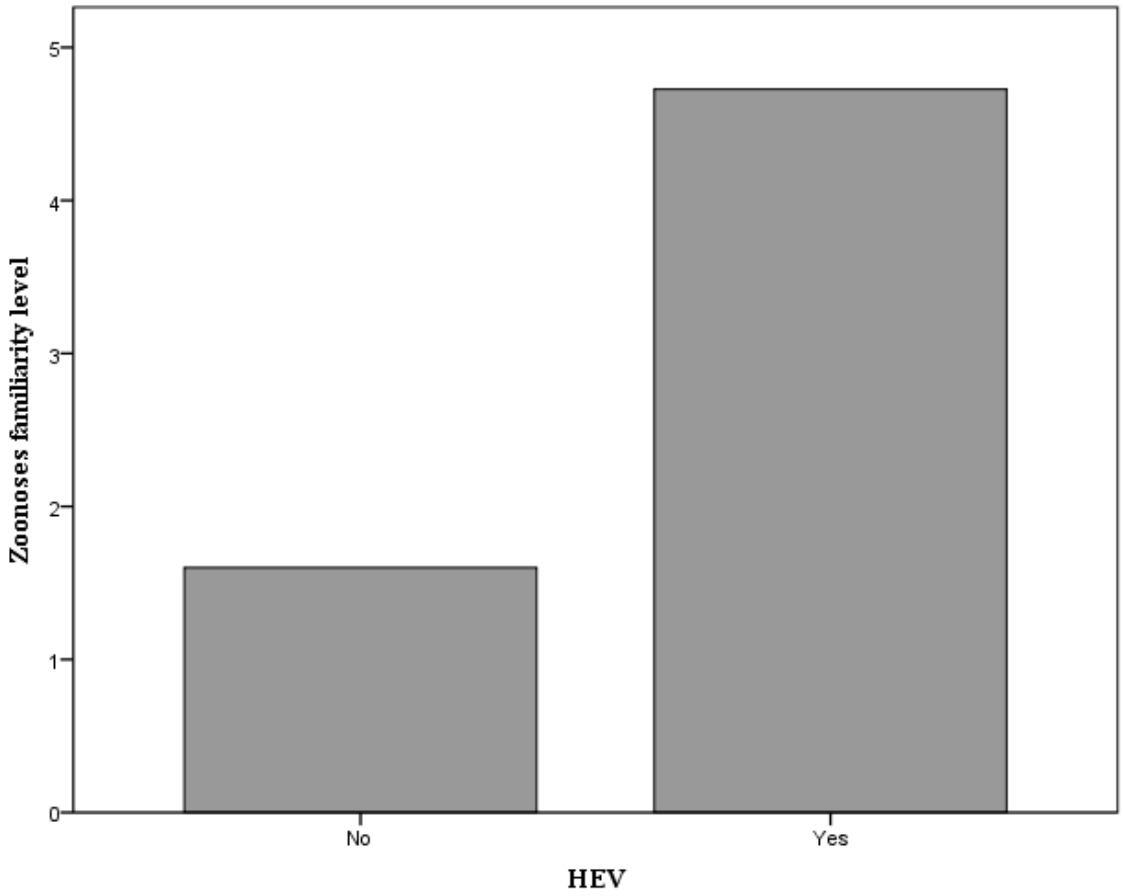

FIGURE 10. HEV Positivity by Owner Zoonoses Familiarity

TABLE 1. Canine Characteristics

Breed	N	Mean Age	% Male	% Mix	# TVC	# PFP	# HF	# HEV +	AKC Group	Animal Contact?
American Blue Heeler	2	2	50%	0%	0	2	0	1	Herd	Animal
Australian Shepherd	2	4	50%	100%	1	0	1	1	Herd	Animal
Basenji	1	2	0%	0%	0	1	0	1	Hound	Animal
Beagle	5	3.8	20%	0%	1	4	0	3	Hound	Animal
Border Collie	9	1.89	67%	100%	0	3	6	3	Herd	Animal
Boxer	6	2.67	83%	67%	1	1	4	2	Work	Non
Chihuahua	13	6.15	46%	8%	2	11	0	7	Toy	Non
Chow	5	3.6	60%	100%	0	1	4	2	No Sport	Non
Cocker Spaniel	4	9.5	0%	25%	4	0	0	1	Sporting	Animal
Coon Hound	1	5	0%	100%	0	1	0	1	Hound	Animal
Corgi	1	6	100%	100%	0	0	1	0	Herding	Animal
Cur	1	2	100%	0%	0	1	0	1	Herding	Animal
Dachshund	7	4.86	86%	29%	3	4	0	1	Hound	Animal
Doberman	1	3	0%	100%	0	0	1	0	Working	Non
English Setter	1	7	0%	0%	1	0	0	1	Sporting	Animal
German Shepherd	7	2.29	43%	86%	2	2	3	1	Herding	Animal
Ger.Shorthair Pointer	2	3	50%	0%	2	0	0	1	Sporting	Animal
Golden Retriever	1	7	0%	0%	1	0	0	0	Sporting	Animal
Great Pyreneese	1	10	0%	0%	1	0	0	0	Working	Non
Greyhound	4	6.25	25%	0%	4	0	0	0	Hound	Animal
Heeler	2	2	100%	100%	0	0	2	0	Herding	Animal
Hound	3	2	0%	100%	0	0	3	0	Hound	Animal
Jack Russell Terrier	2	7	100%	100%	1	0	1	0	Terrier	Animal
Lab	32	3.8	56%	56%	11	8	13	13	Sporting	Animal
Maltese	1	2	100%	0%	0	0	1	0	Toy	Non
Mastiff	1	3	100%	100%	1	0	0	1	Working	Non
Papillion	1	7	0%	0%	1	0	0	0	Toy	Non
Pittbull	7	2.57	43%	71%	0	0	7	1	Terrier	Animal
Pointer	1	4	0%	100%	0	0	1	1	Sporting	Animal
Rat Terrier	1	9	100%	0%	0	1	0	1	Terrier	Animal
Red Heeler	1	13	100%	0%	1	0	0	0	Herding	Animal
Rottweiler	1	2	0%	100%	0	0	1	0	Working	Non
Saluki	1	4	100%	0%	1	0	0	0	Hound	Animal
Schnauzer	5	4.6	40%	20%	2	2	1	2	Working	Non
Sharpei	1	3	0%	100%	0	0	1	0	No Sport	Non
Shihtzu	1	2	100%	100%	1	0	0	1	Toy	Non
Springer Spaniel	1	3	100%	100%	1	0	0	0	Sporting	Animal
Terrier	4	7.25	25%	100%	2	2	0	2	Terrier	Animal
Yorkshire Terrier	2	9	0%	0%	1	1	0	2	Toy	Non
Undetermined	2	6.5	50%	100%	1	0	1	1	--	--
Total	**144**	**4.32**			**47**	**45**	**52**	**52**		

TABLE 2. Canine Contact with Other Animals*

Type of Animal	Rarely/Occasionally	Often/Frequent	Never/NA
Cattle	5	2	40
Goats	3	0	44
Sheep	3	0	44
Horses	6	0	41
Pigs, Domestic	3	0	44
Chickens	4	2	41
Ducks	4	0	43
Other Fowl-Dom	7	2	38
Deer	3	0	44
Antelope	3	0	44
Wild Hogs/Pigs	5	0	42
Water Foul	3	0	44
Turkey	3	0	44
Freshwater Fish	3	0	44
Rabbits	4	1	42
Totals	59	7	639

*****Totals are greater than the number of canines surveyed as owners indicated more than one answer.

TABLE 3. Canine Outdoor Experience*

Yard Type	Never Out	Out for Bathroom	Occasionally	Most of Day	Outside Only
Inside Only	0	5	7	3	0
Fenced Yard	0	7	22	13	0
Fenced Farm	2	1	1	11	0
Tie Out	2	0	0	0	0
Free to Roam	2	0	2	0	1
Totals	6	13	32	27	1

*****Totals are greater than the number of canines surveyed as owners indicated more than one answer.

TABLE 4. Canine Grooming Experiences*

Groomer	Rarely/ Occasionally	Often/ Frequent
Professional	17	3
Friend	0	0
Family	1	1
Yourself	9	6
Never	6	2
Totals	33	12

A total of 9 owners indicated that their dog was never groomed.

* Totals are greater than the number of canines surveyed as owners indicated more than one answer.

TABLE 5. Results of Yes/No Survey Questions

Question	Yes	No	NA
Is canine fed raw meat	1	45	1
Is canine allowed chew toys/treats	33	11	3
Does canine have contact with other canines	31	14	2
Is canine a working animal	4	27	16
Does the owner take the animal for walks	25	18	4
Does the owner allow the canine to lick them	34	6	7

TABLE 6. P Values from Statistical Analyses of Canine Contact or Characteristics

Question	Human Knowledge†	Human Familiarity - Zoonoses†	Human Import. Vacc.†	Human Concern Contracting†	Canine Age†	K9 HEV +‡
Gender	0.173	0.453	0.763	0.890	0.583	0.241
Is given chewtoys	0.305	0.566	0.876	0.390	0.104	0.703
Owner has other canine	0.093*	0.353	0.753	0.247	0.010**	0.134
Contact w/other canines	0.153	0.285	0.480	0.931	0.067*	0.448
Contact w/domestic	0.409	0.254	0.421	0.081*	0.154	0.264
Contact w/wild animal	0.145	0.009**	0.350	0.000**	0.010**	1.000
Contact w/animal	0.836	0.312	0.421	0.081*	0.154	0.458
Is a working dog?	0.195	0.055*	0.864	0.479	0.229	0.285
Taken for walks?	0.020**	0.244	0.121	0.411	0.868	0.476
Allowed to lick?	0.363	0.006**	0.281	0.580	0.526	0.169
Professionally groomed	0.260	0.061*	0.771	0.619	0.665	0.716
Groomed at all	0.731	0.929	0.593	0.251	0.175	0.545

**p value is significant ($p<0.05$)
*p value is marginally significant ($0.10>p>0.05$)
† T test
‡ Chi Square

TABLE 7. Multivariate Logistic Regression Factors Associated with HEV Infection in Clinic Canines (n=47)

	Unadjusted P-value	Unadjusted Odds Ratio (95% C.I.)	Adjusted P-value	Adjusted Odds Ratio (95% C.I.)
Mix/Purebred	.426	.377 (.03 - 4.16)	N/A	
Owner Knowledge	.974	1.016 (.40 - 2.57)	N/A	
Owner Familiarity	.322	1.602 (.63 - 4.07)	.044**	1.666 (1.02 - 2.73)

**p<.05, after 3 steps

TABLE 8. Multivariate Logistic Regression Factors Associated with HEV Infection in All Canines (n=143)

	Unadjusted P-value	Unadjusted Odds Ratio (95% C.I.)	Adjusted P-value	Adjusted Odds Ratio (95% C.I.)
Mix/Purebred	.357	.619 (.22 - 1.72)	N/A	
Site				
Holding facility	[reference]			
Shelter	.000**	21.003 (5.55- 79.54)	.000**	15.500 (4.98 – 48.26)
Private clinic	.005**	5.203 (1.63 – 16.64)	.008**	4.183 (1.46 – 12.01)

**p<.05, after 2 steps

CHAPTER FIVE: DISCUSSION

Seroprevalence Results

This thesis sought to answer four questions: 1. Is evidence of HEV infection found in canines in Smith County? 2. Is there any demographic parameter that makes canines more likely to be positive? 3. What do involved human owners know and/or believe about zoonotic diseases? and, 4. Is there a link between owner's cognitions or canine's environment and HEV positivity?

Based on the data reported herein, the first question has a definitive answer - there is evidence of HEV infection in canines in Smith County, Texas. With 52 positive canines, corresponding to a seroprevalence of 48%, these results were surprising. This study's rate almost doubled that of previous studies. In previous literature, the highest reported seroprevalence rate for canines was 27% in Vietnam (Tien *et al.*, 1997). Even if all the undetermined tests were negative, a 36% positivity rate (of the original 144 samples) is still a 44% increase over the highest previously reported study (Tien *et al.*, 1997). What is it about Smith County that leads to such high seroprevalence in canines? Could 48% prevalence be considered a new norm for Northeast Texas, or even all of Texas? And is HEV confined to this area, or is there evidence of infection across the rest of the US?

It was assumed that facility type would play a significant role in positivity. Presuming that swine are reservoirs and that genotypes 3/4 HEV could be spread

enterically through contaminated food and water, like genotypes 1/2, it was believed that the holding facility would have the highest number of cases since those canines were more likely to have been free to roam in areas where wild pigs and hogs (which are considered a nuisance in Texas [Murphy, 2013]) live. These canines also would be those most likely to consume food and water of questionable, possibly contaminated, quality. The assumption that human involvement would result in lower positivity was incorrect. Those animals that had some human owner investment – even if only to drop them off at a shelter – were significantly more likely to be HEV positive. But even that finding was at odds with what would be assumed if the location probability were simply reversed. Whatever this factor is though, it is not about poor living conditions, nor is it about mere human ownership. What else could it be?

Any number of circumstances at the shelter, the site with the highest prevalence, could have resulted in the number of positive cases. For instance, many of the cages were constructed of chicken wire, implying access to the outside. Some cages were even built on dirt – perhaps a source of the HEV. The animals were all walked, cleaned, fed, watered, and provided veterinary care by the same set of humans – a potential for cross-contamination. Could one, or all, of the workers/volunteers have picked up the virus from one cage and inadvertently spread it throughout the rest of the animals in the facility? This would account for why both those animals who lived primarily inside as well as outside at the shelter were positive. Or possibly, since the animals were all exercised in the same yard, they could have been infected at that point of contact. The infection could have come from swine that previously had access to that area – as the shelter recently expanded. An infected dog could have contaminated the yard, or perhaps another

mammal could have introduced the contagion into the play yard and thus the shelter canines. Yet another possibility exists in that shelter animals are exposed to the most varied number of humans of any of the animals we tested. In hindsight, the researcher questions whether pre-sorting by these locations was a salient design idea since IgG production takes several weeks and two of the sites – the holding facility and shelter – imply a sudden change in the canine's exposures.

Still, could humans or human environments be the transmission vector to canines? A recent study in Louisiana found that rabbits bred for medical testing were positive for HEV, yet their environment was clean. This article surmised that their human handlers may have introduced the infection into that control group (Birke *et al.*, 2014). Likewise, could humans have introduced and spread HEV through the shelter canine pack? Further tests, along with defining the transmission cycle of HEV, would be needed to determine which index case came first, the human or the canine.

Could another possible route of infection be through contaminated dog food? Infection is not likely due to canned food consumption, in this study, because the large majority of owners did not give wet food to their pets. Thus the questions emerge, Could dry food be the infection source? To what extent are pork products found in dry food? and, What safety measures are used to test these ingredients?

Yet another surprise was that purebred canines were significantly more likely to be positive than those of mixed breed. The researcher assumed that mixed breeds would be more likely to be positive. This belief stemmed from the assumption that those who owned purebreds would have invested heavily in them, thus would be less likely to expose the animals to possible sources of infection; yet with 56% of the total number of

positive cases being purebred, obviously this assumption was incorrect. Perhaps those that were purebred were trained better thus resulting in being taken to riskier environments, whereas mixed breed dogs did not have as extensive training and their human owners were less comfortable taking them places where they may have been infected. Another possibility is that purebreds were purchased for the purpose of some work specialization that would expose them, and while no significant association was found between HEV and those reported as being working canines (Table 6), it is interesting to note that of the four canines reported as being working animals, three of them (75%) were positive.

Another possibility to account for the association between purebreds and being positive is that some animals may have been erroneously reported as purebred. Of the total 64 animals marked as purebred, 44 (69%) were from either the shelter or the holding facility. For the shelter animals, breed was indicated on their surrender card and verified by the veterinarian working with this study. Thus the information on whether the animals were purebred could have been inaccurate since the determination was based on what was reported by the surrendering party, the shelter veterinary technician, or the veterinarian working on this study. For those animals from the holding facility, the determination of breed and, by extension, whether or not the animal was a mix was solely made by the study veterinarian – based upon physical characteristics – which could also introduce error into the determination of either breed or genealogy (being mixed or purebred). Additionally, the majority of the animals from the clinic (57%) were adopted or found, thus introducing another potential for misreporting. Furthermore, even animals from breeders or pet stores could derive from mixed breed, at some point in their

genealogy. So whether or not the animal is purebred may be a poor inclusion factor for HEV positivity.

One outcome that seemed to follow the researcher's logic was the fact that those canines that fell into the animal contact group had a higher prevalence than those whose AKC group was historically not known for having contact with animals (Table 1). With 34 (65%) of the positive canines belonging to the animal contact group, there appears to be a marked difference between contact groups though no significance was found between contact group and positivity ($p=0.331$). The high proportion of positive contact animals could be due to a number of factors. The animal contact breeds may be more curious – thus more likely to come into contact with contaminated material than their non-contact counterparts. It could also be that they are more attractive to owners, since many of the individual breeds within the animal contact group are reportedly easy to train and great family pets (AKC, 2014). These speculations however are contingent upon there being some transmissible pathway from humans to dogs. On the other hand, it could be the popularity of those breeds (e.g. Labradors and Beagles) within the animal contact group resulting in inexperienced owners owning them and allowing them to come into contact with contaminated material due to inability to control the animal.

Again, the researcher was surprised by the outcome of what the human owners knew about zoonotic diseases. While the overall score of the knowledge section was low, 1.3 out of 6 possible questions, the responses to several individual questions were quite surprising. The highest correct response rate (86% correct of 36 individuals) was on the question regarding the prevention of infectious disease spread (washing ones hands). However, it was the response to a question regarding hepatitis in general that was the

most surprising: While only 1% of individuals knew which types of hepatitis there are currently, 72% of them knew that hepatitis primarily affected the liver. This far exceeded what the researcher expected and was encouraging. The fact that human owners have general knowledge of what hepatitis does will make it easier, should the need arise, to appraise or educate the general public about risk factors as well as symptoms of HEV.

On the other hand, the surprisingly high number of individuals who knew that hepatitis affects the liver was counterbalanced by the responses to familiarity and concern questions. While most owners believed that canine vaccinations were very important (average of 9.2 out of 10), the average familiarity of the owners with zoonotic diseases was low.

The final question this thesis attempted to answer involved whether or not a link existed between the owners' cognitions and whether or not their pets were positive for anti-HEV bodies. Again, the results were surprising. A significant association was found between those with higher scores on knowledge and the positive canines. Additionally, those who indicated a higher familiarity with zoonotic diseases were also more likely to have positive animals.

While no individual behavior was associated with being HEV positive, it does make one wonder as to whether or not those owners more familiar with zoonotic diseases, and likely more educated in general, were more likely to introduce their animals into new environments and to other canines, thus more likely to encounter or spread HEV. However, this seems to be contradicted by the finding that for the two behaviors with significant association between the behavior and knowledge, it was found that the higher knowledge score was associated with not walking your animal and/or owning

more than one canine. Another speculative possibility is that those owners more acquainted with zoonotic diseases, thus able to recognize disease in canines, would be more likely to give up their pet if they detected signs of disease. However, this line of thought seems most unlikely.

Limitations and Strengths

This study had several limitations. The first set of limitations stem from the study design. While the sample size was sufficient to meet the specified power for general prevalence, a larger sample size, and/or selecting canines from many locations, would have provided better generalizability. Still, the ability to capture HEV information in animals from these different sites is unprecedented. The study may have provided a more complete picture had the researcher tested all canines, regardless of age, instead of the lower limit of one year. This study was originally intended to only test those animals whose immune systems were mature, yet perhaps infection is higher in younger canines as it seems to be in swine – the younger the animal is, the higher the HEV viremia (Fernandez-Barredo *et al.*, 2006). Another limitation of the study design was the survey questions. If there had been additional, more in-depth questions on owner's knowledge of zoonoses, the researcher may have had a better picture of what was known in owners, in order to find more in-depth correlations between knowledge and HEV positivity. Additionally, if questions about the human owner's habits, including eating habits and educational background, had been included, more inferences may have been drawn. On the other hand, including more questions could have resulted in fewer participants filling out the entire questionnaire. As it was, 11 of the 47 owners (23%) did not answer any

part of the knowledge section. Even so, these responses were enough to make some preliminary conclusions.

Additional limitations resulted from actual sample collection. For any animal which did not have readily available demographic information (from previous or current owners), the veterinarian consulting with this study made the final determination regarding both age and gender – this could have introduced error. However, by using the same veterinarian for all collections, and given the degree of training veterinarians have in age estimation using tartar buildup on teeth, as well as determining breed, any bias was offset by reliability. One further limitation of the collection process was that the selected canines were pseudo-randomized. That is, due to a smaller number of animals on site at any one moment than originally anticipated, as many canines as possible were sought at that moment (convenience). On the other hand, randomization did occur in that, with the exception of PFP, the specimen collecting was over the course of 1 to 3 weeks – allowing a much more varied number of animals to arrive randomly and be available.

In regard to laboratory processing, a limitation for this study was the ELISA kit. The practice runs and initial run showing 100% positivity could be due to any number of factors including errors in collecting the specimen, human error while running the assay, equipment error, and/or contaminated or faulty assay kits due to questionable lab results when run on-site. The collection process was discounted as the laboratory problem because the specimens were collected by the same licensed veterinarian in the same style and lot of tubes. Additionally, the tubes had no additive, so there was no potential for contamination at that point. The assay was performed following the manufacturer's instructions and supervised by a faculty member from UT Tyler's Biology Department –

again, a very unlikely source of error. This left the possibility of either the equipment being used or the assay kit itself being faulty. Unable to determine which might have been the issue, the samples were sent to the ELISA kit manufacturer for analysis. While this could be a limitation, as no additional test for validity have been completed at this point, it is a strength that the lab company stood by its product by offering to run the analyses. An added limitation for this study was that some of the samples hemolyzed (24%) causing indeterminable readings; but, enough readable samples were returned to match what our original projections for sample size were.

Additional limitations stemmed from lack of funding. A larger sample size, a more extensive survey, human specimen collections, RT-PCR analyses, etc. would all have been desired, and accomplished, had funding not been an issue. One further limitation was time. This study had to be completed during the course of a two year graduate degree program. If this research was not organized around a student's program requirements, timing would have been less of an issue as more time would have been available for research activities. Nonetheless, 144 samples and demographic information were collected and analyzed.

While the study design had limitations, it was also one of the greatest strengths. Namely, this study made use of animals from three different parts of Smith County – giving the researcher as wide a range of animals and environments as possible. Further, all blood draws were performed by the same person, all sample preparations were performed by the same two people, and all analyses were performed by the same laboratory – thus accounting for any discrepancy in technique between individuals. The participation rate for the human owners was 100% – which lends validity as the

individuals who consented to participate were not compensated in any way for their participation (even though not every owner answered every question) – again giving us a broad range of life experiences. Lastly, every aspect of the design was vetted by professionals in their respective fields, including a board licensed veterinarian, a professor of biology, the hepatitis expert from the Texas State Department of Public Health, and an infectious disease epidemiologist and health professor.

Discussion and Further Studies

While this study answered four questions, it gives rise to many others: Is it HEV genotypes 3/4 we are seeing? What is the origin of HEV in this area? How is it being spread? How far has it spread? Is it truly related to human behavior and cognition? Does it pose a real risk? And, perhaps most important, if it is pathogenic, how can one prevent it?

One of the major questions facing researchers today is the origin and transmission route of HEV. Swine are already established as reservoirs, but the majority of humans have no direct contact with swine – and thanks to current public health education campaigns, many individuals know to handle raw pork products with care – cooking thoroughly and washing hands and surfaces after preparing the pork. This raises the recurring question, how are humans contracting the infection (see Texas human cases, Amon *et al.*, 2006, Tohme *et al.*, 2009)? The assumption behind this study is that, due to the widespread appeal of canines, canines themselves could be a transmission vector. In addition, knowing that Texas has a feral pig/hog problem (Murphy, 2013) and knowing that many canines have the potential to come into contact with some form of contaminated material (assuming that infected feral pigs in the area are shedding active

virus), the finding that those from the holding facility, the ones that, most likely came into contact with swine or swine environments (but had the lowest proportion of HEV prevalence) calls that belief in to question. Perhaps the idea that these canines have the chance to roam implies they are less likely to be infected because they are not continuously exposed to the virus. Perhaps those canines with somewhat steady human contact are more likely to have HEV because they are confined to a specific area that could have been contaminated – by birds, rodents, or even man. While it is surmised that swine are a reservoir, perhaps any domestic animal can be the transmission vector. After all, one theory is that all of the diseases that face mankind today stem from the domestication of animals (Diamond, 1998).

For instance, according to another theory, horses have been established as the main incubator of the Spanish Influenza pandemic that killed between 20 and 40 million people at the beginning of the 20th century (Billings, 1997; Knox, 2014). Also, a precursor to West Nile Virus cases in humans is the detection of West Nile Virus neutralizing antibodies in horses (Gould & Fikrig, 2004). Should we look to horses for answers to HEV transmission? After all, there are an estimated 3,000 to 9,000 horses in Smith County (Wittich, Ward, Fosgate, & Srinivasan, 2008). On the other hand, only six of the animals from the veterinary clinic in this study were reported as having contact directly with horses, of which only one was HEV positive. Further study clearly needs to be done on other potential reservoirs or vectors.

Another issue raised by this study questions a third species, humans, and is a true 'chicken or egg' conundrum. To reiterate – canines with regular human contact were more likely to be positive. This and the fact that small canines – those commonly thought

of as lapdogs – were also positive; perhaps attention needs to be turned towards humans. Is it possible that human beings contracted the disease through some other source, and are spreading HEV to canines? Humans constantly are coming into contact with items that canines ingest or chew on. Whether preparing their animal's food, petting them, playing with them, or even handling their toys – is contact with humans how canines are contracting this infection?

The idea of humans as transmission vectors seems highly unlikely for genotypes 3/4. Assuming that genotypes 3/4 evidence a similar mortality rate as 1/2 and person to person transmissibility is possible, HEV would have come to the forefront of epidemiological attention long before now and would have prompted far more scrutiny. Another idea to consider is that canines are not able to transmit the virus. This would explain why no one has been able to isolate RNA from HEV positive canines (Liu *et al*., 2009; Arankalle *et al*., 2001; Mochizuki *et al.*, 2006). Both scenarios would need further study in order rule them out completely.

Another scenario to consider is the geographic shift of genotypes – could genotypes 3/4 HEV migrate into developing countries and could genotypes 1/2 move to developed? It is entirely possible that genotypes 3/4 have been in developing countries all along, but only the widespread human strains have come to light because, unless it is causing animals to die *en masse*, animal disease will not be sought. It is also possible that humans in developing countries have genotypes 3/4 (whether a co-infection or not) but no one has identified the strain – probably due to limited resources. Being able to fully track the reach of HEV genotypes 3/4 would add to the body of knowledge.

There is incomplete knowledge about HEV genotypes 3/4: Where does it come from, how is it spread, and when compared to genotypes 1/2, how deadly is it? It would stand to reason that 3/4 would match 1/2 in mortality rates, but until more cases are identified it will be difficult to accurately express a 3/4 mortality rate. We know genotypes 1/2 affect immunocompromised persons. The death toll could be quite high were there to be a full scale outbreak in a developed country such as the US. The reasoning behind this idea includes the notion that the US healthcare system contends largely with chronic diseases – like HIV, cancers or diabetes. For example, cancer survivors occasionally receive chemotherapy which suppresses the immune system. Furthermore, diabetes shows special association for infectious diseases like tuberculosis (Jeon & Murray, 2008), so why not HEV? Aging populations naturally tend to be immunocompromised anyway, so, if genotypes 3/4 mimic 1/2, and infection reaches a large number of chronic disease survivors it would result in a high number of deaths. If HEV 3/4 is as deadly to immunocompromised persons as 1/2, then the general populace needs to be made aware of HEV and policy needs to be framed to keep humans safe if HEV outbreaks emerge. If this scenario plays out then both a commercially available rapid diagnostic test and vaccines for both humans and animals need to be created and approved.

Hepatitis E detected in humans is now on the list of reportable diseases in Texas (Texas Department of State Health Services, 2014), when, at the beginning of this study, it was not. Why the sudden inclusion? On one hand, zoonotic strains of HEV have begun to appear in large outbreaks. For example, an outbreak of genotype 4 occurred in France in 2011. There were no fatalities associated with this outbreak, but over 200 individuals

were identified as HEV positive (Jeblaoui *et al.*, 2013). On the other hand, individual sporadic cases of HEV are found throughout the world. This coupled with the holes in knowledge regarding transmission as well as mortality means that governments will want to keep a close eye on any cases to try and determine patterns. Authorities will want to focus their resources on those diseases of the most import, i.e. those with the highest severity or transmissibility.

The common theme to all these speculations is that more studies are warranted to enhance our understanding of HEV reservoirs and transmission patterns. While this study explored only one aspect in one area of HEV research, a thorough undertaking of the complete transmission cycle would shed much needed light on the entire viral ecology. More specifically, regarding canines, larger studies involving more owned animals would be essential as well as identifying the strains of positive canines. Additionally, being able to link individual canines to their humans through both ELISA and molecular analysis using RT-PCR would lead to a more definitive theory regarding transmission. Studies on other animals, for instance wild and domestic birds as well as horses and other domesticated mammals, would shed more light on the prevalence and transmission pathways of HEV infection. Moreover, studies on commercially available meat, such as those conducted on pork processing plants as well as points of sale (Feagins *et al.*, 2007; Berto *et al.*, 2012; Berto *et al.*, 2013), may shed light on the transmission cycle especially given the results of the French outbreak from which infections were traced back to contaminated liver sausage (Jeblaoui, *et al.* 2013).

CHAPTER SIX: CONCLUSION

Hepatitis E came on the scene within the last 50 years. Along with countless other diseases, HEV can be traced, in part, to animals. Human beings have created the perfect storm for some of these emerging diseases; with the ever-expanding and mobile human population, and with areas of high population density, low sanitation, and overall civic strife in addition to the encroachment of humans into wild animals' territories, humans are being exposed to a widening array of infections (e.g. Ebola, Lyme disease, and Hantavirus). Hepatitis E has been steadily moving into our own backyard, and in order to prevent it we have to know more about it. This research found 48% of canines to be positive, but this study is but a stepping stone on the path to truly understanding, and thus combating, this infection. This study is the first in the US to attach importance to canines as possible infection vectors, as well as the first study to associate human cognition to pet canine behaviors and subsequently, both cognition and behavior as risk factors for HEV infection. Additionally, evidence from this study could lead to the establishment of canines as a new reservoir for HEV. And while HEV may seem innocuous at first glance, all great plagues begin with a single infected person.

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APPENDIX A: IRB and IACUC Approvals

The University of Texas at Tyler
Institutional Review Board

October 26, 2013

Dear Ms. Grzybowski,

Your request to conduct the study: *Hepatitis E Seroprevalence Feasibility in Canis lupus familiaris in Smith County, Texas,* IRB #F2013-29 has been approved by The University of Texas at Tyler Institutional Review Board under expedited review. This approval includes a waiver of written informed consent and your assurance of participant knowledge of the following prior to study participation: this is a research study; participation is completely voluntary with no obligations to continue participating, with no adverse consequences for non-participation; and assurance of confidentiality of their data.

In addition, please ensure that any research assistants are knowledgeable about research ethics and confidentiality, and any co-investigators have completed human protection training within the past three years, and have forwarded their certificates to the IRB office (G. Duke).

Please review the UT Tyler IRB Principal Investigator Responsibilities, and acknowledge your understanding of these responsibilities and the following through return of this email to the IRB Chair within one week after receipt of this approval letter:

- This approval is for one year, as of the date of the approval letter
- Request for Continuing Review must be completed for projects extending past one year
- Prompt reporting to the UT Tyler IRB of any proposed changes to this research activity
- **Prompt reporting to the UT Tyler IRB and academic department administration will be done of any unanticipated problems involving risks to subjects or others**
- Suspension or termination of approval may be done if there is evidence of any serious or continuing noncompliance with Federal Regulations or any aberrations in original proposal.
- Any change in proposal procedures must be promptly reported to the IRB prior to implementing any changes except when necessary to eliminate apparent immediate hazards to the subject.

Best of luck in your research, and do not hesitate to contact me if you need any further assistance.

Sincerely,

Gloria Duke, PhD, RN
Chair, UT Tyler IRB

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December 2, 2013

Dr. William Sorensen, Associate Professor
Department Health and Kinesiology
Campus

Dear Dr. Sorensen:

Your research protocol "**Hepatitis E Seroprevalence in *Canis lupus familiaris* in Smith County, Texas**" has been approved as an initial review and assigned IACUC protocol #111.

Please acknowledge your understanding of the following responsibilities through email to the IACUC committee within one week after receipt of this letter:

- This protocol will be active for three years. A continuation request must be submitted to the IACUC for an extension through the one-year date before the study can be continued. **Your request for continuation review will need to be submitted and approved by IACUC by December 2, 2014.**
- Prompt reporting to the IACUC of any proposed changes to this teaching protocol.
- Prompt reporting to the IACUC and academic department administration of any unanticipated problems involving risks to animals or persons.
- Suspension or termination of protocol may occur if there is evidence of any serious or continuing noncompliance with federal, state, local or UT Tyler regulations or any aberrations in original proposal.

Best wishes for success in your research endeavor.

Sincerely,

Lance R. Williams, Ph.D.
Chair, UT-Tyler IACUC

APPENDIX B: Letters of Support

INDEMNIFICATION AGREEMENT BETWEEN DR. WILLIAM SORENSEN (REPRESENTATIVE OF THE DEPARTMENT OF HEALTH AND KINESIOLOGY AT THE UNIVERSITY OF TEXAS AT TYLER) AND SMITH COUNTY SHERIFF'S OFFICE (AS REPRESENTATIVE OF SMITH COUNTY)

AGREEMENT:

In exchange for access to the Smith County dog impound facility and cooperative assistance with regard to a veterinary or scientific study being conducted by Dr. William Sorensen or his staff or collaborators with the Department of Health and Kinesiology at the University of Texas at Tyler (hereinafter collectively referred to as "Sorensen Study Participants"), agree, to the fullest extent permitted by law, to indemnify and hold Smith County, Texas and her agents, representatives, and/or employees harmless from any and all claims for or entitlement to any damages arising from claim(s) relating to the below-described project, including but not limited to out-of-pocket, special, incidental, indirect, or consequential damages arising out of, resulting from, or in any way related to the project described below. Sorensen Study Participants expressly agree that they are assuming all risk with regard to the project described below and shall reimburse Smith County for all damages, including court costs and attorney's fees for the collection of such reimbursement, if necessary.

Smith County shall receive public benefits for health and promotion of a health and safety study.

Smith County does not waive any sovereign immunity under this agreement.

This agreement is subject to Texas law with venue in Smith County, Texas.

PROJECT:

Hepatitis E Seroprevelance in Stray and Owner Dogs in East Texas, which will help define prevelance of Hepatitis E in canines in East Texas as well as possible biological or epdemiological links in dog/owner pairs.

Accepted by and for Smith County, Texas:

BY: _____
Sheriff's Office

William Sorensen
William Sorensen

10/12/2013
(Date Signed)

William Sorensen
(Project Participant - Printed)

10/12/2013
(Date Signed)

BY: William Sorensen
(Signature)

William Sorensen
(Signature)

SUBSCRIBED AND SWORN TO BEFORE ME by the said WILLIAM SORENSEN on this the ___ day of October, 2013.

_____
Notary Public in and for the
State of Texas

SUBSCRIBED AND SWORN TO BEFORE ME by Project Participant _____ on this the ___ day of October, 2013.

_____
Notary Public in and for the
State of Texas

From: Gayle Helms [mailto:gayle@hsoet.org]
Sent: Wednesday, May 29, 2013 4:14 PM
To: William Sorensen
Subject: RE: request for collaboration

Dr. Sorensen:

I am interested in a collaboration with UT Tyler on this study; however, I have a couple of questions.

- Will dogs from other shelters be included in this study?
- What is the sample size of each blood draw from each dog?
- Are there any health risks for our dogs?

Thank you,

Gayle Helms,
Executive Director
Pets Fur People
Like us on Facebook, Twitter and Pinterest

Tyler Veterinary Center
4505 Old Bullard Rd.
Tyler, TX 75703
(903)-581-2070

May 30, 2013

William Sorensen, PhD
Department of Health and Kinesiology
University of Texas at Tyler
3900 University Blvd.
Tyler, TX 75799

RE: Letter of Support

Dear Dr. Sorensen:

As a practicing veterinarian in Tyler, I am pleased to collaborate with you on the proposed project "Hepatitis E Seroprevelance in Stray and Owner Dogs in East Texas". This project will help to define the prevelance of Hepatitis E in canines in the East Texas area. In addition, this project will help to identify possible biological or epidemiological links in dog/owner pairs.

My contribution will be to help obtain avenues to identify stray and owned dogs in this area for testing as well as performing the blood draws for testing. I am excited to help collect these specimens for testing and observing the following test results.

I believe this project will help to bring to light the possible zoonotic link between dogs and humans with Hepatitis E. I am looking forward to participating in this project with you and the other investigators.

Sincerely,

Sharon Phillips DVM

Sharon Phillips DVM
Tyler Veterinary Center

APPENDIX C: Educational Flyer

About the Disease

- ⇒ It is a viral infection that is usually short lived
- ⇒ Less than 0.01% are in danger of dying from this disease (if not immunocompromised)
- ⇒ Symptoms are usually very mild, if any appear at all
- ⇒ The classical known transmission route is through contaminated water
- ⇒ There is no known cure and no approved vaccine

Possible Prevention

- ⇒ Don't eat anything that hasn't been washed first or cooked thoroughly
- ⇒ Don't drink anything/ use ice cubes made with water from unclean sources
- ⇒ Wash your hands often

Hepatitis E

What You Should Know

Extra Precautions

For those with weakened immune systems (pregnant women, cancer patients receiving chemo, HIV/AIDS positive persons, etc.)

- ⇒ Be extra careful when handling dog waste – always wash your hands afterwards with hot, soapy water
- ⇒ If you think you may be sick, contact your family physician for a check up

What Not to Do

- ⇒ Do Not panic-your chance of getting it is very slim
- ⇒ Do Not get rid of your dog-there is no known Hepatitis E link between dogs and humans

Remember: by practicing good hand hygiene, you and your dog can live a long, happy life together.

APPENDIX D: Survey

Survey for Dog Owners

All information gathered will remain anonymous and confidential

The following questions pertain ONLY to the dog you have brought in today. Please mark the best answer to each question; for questions where you are asked to fill in the blank, please write legibly. If you are unsure as to what the question is asking, please ask the administrator.

Gender: ☐ Male ☐ Female **Age** *(at last birthday)*: _____ **Purebred**: ☐ Yes ☐ No

Breed (*if mixed, most predominant*): _____

Has the dog been spayed/neutered (*fixed*)? ☐ Yes ☐ No ☐ Unknown

How long have you owned this dog? _____ **How old was the dog when you got it?** _____

In what city/town does the dog live? _____

Where did you get the dog from? ☐ Breeder ☐ Pet Store ☐ Pet Seller ☐ Craigslist Ad ☐ Adoption ☐ Friend/Family Member ☐ Found the Dog.

Does your dog eat raw meat? ☐ Yes ☐ No.

If Yes, what type of meat? _____

If No, what type of dog food do you use? _____

Does your dog get chew toys/treats? ☐ Yes ☐ No.

If Yes, what type (antler, plastic, rawhide, etc.): _____

Do you own other dogs? ☐ Yes ☐ No. **If yes, how many?** _____

Does your dog have contact (*more than 15 minutes*) **with other dogs NOT owned by you** (*e.g. dog park, kennels, obedience classes, parks*)? ☐ Yes ☐ No

If yes, how often does your dog have contact with other dogs:

☐ Rarely, once or twice a year
☐ Occasionally, once or twice a month
☐ Often, once or twice a week
☐ Frequently, nearly every day

Does your dog have contact (*more than 15 minutes*) **with the following *domestic* animals and/ or their living areas** (*check all that apply for each animal, if the answer is none, leave the row blank*).

	Rarely *once or twice a year*	**Occasionally** *once or twice a month*	**Often** *once or twice a week*	**Frequently** *almost every day*
Cattle				
Goats				
Sheep				
Horses				
Pigs				
Chickens				
Ducks				
Other Fowl				

Does your dog have contact (*more than 15 minutes*) **with any of the following *wild* animals** (*check all that apply for each animal, if the answer is none, leave the row blank*)

	Rarely *once or twice a year*	**Occasionally** *once or twice a month*	**Often** *once or twice a week*	**Frequently** *almost every day*
Deer				
Antelope				
Wild Hogs/Pigs				
Water Foul				
Turkey				
Freshwater Fish *(any type)*				
Rabbits				

Is your dog a working dog (*e.g. hunting, herding, service)*? ☐ Yes ☐ No. **If yes, type?**_____

Which of the following best describes your dog's normal outdoor experience (*check all that apply for, if the answer is none, leave the row blank*)**:**

Type of Yard:	**Never Outside**	**Only Outside for Bathroom**	**Occasionally Outside**	**Most of Day Outside**	**Outside Only**
Inside Only Dog					
Own, Fenced Yard					
Own, Fenced Pasture/farm					
Tie Out/Electric Fence-Non fenced area					
Free to Roam					

Do you take your dog for walks? ☐ Yes ☐ No **If yes, indicate all areas you take your dog walking:** ☐ Streets/sidewalks ☐ Parks ☐ Marked Trails ☐ Dog Parks ☐ Woods ☐ Beaches

Do you allow your dog to lick you? ☐ Yes ☐ No

Indicate how often and who grooms your dog (*check all that apply, if the answer is none, leave the row blank*)**:**

	Rarely *once or twice a year*	**Occasionally** *at least once a month*	**Often** *more than once a month*	**Frequently** *almost every week*
Professional Groomer				
Friend/Charity				
Family Member				
Yourself				
Never Groomed				

Continued on Next Page

The following questions pertain to your general knowledge.
Please do not seek others for the answers.

On a scale of 1 to 10, with 1 being completely unfamiliar and 10 being very familiar, how familiar with zoonotic diseases are you? (*please circle your choice)*

1 2 3 4 5 6 7 8 9 10

On a scale of 1 to 10, with 1 being completely unimportant and 10 being very important, how important are canine vaccinations to you (regardless of whether they are required or not)? (*please circle your choice)*

1 2 3 4 5 6 7 8 9 10

On a scale of 1 to 10, with 1 being not concerned at all, and 10 being extremely concerned, how concerned are you about contracting a zoonotic disease? (*please circle your choice)*

1 2 3 4 5 6 7 8 9 10

For the following, please choose the best answer, if you do not know, mark the box "I do not know"

A zoonotic disease is:

- ☐ A disease only found in zoo animals
- ☐ A disease only found in animals, not humans
- ☐ A disease one gets only from zoos
- ☐ A disease spread from animals to humans
- ☐ A disease spread from plants to humans
- ☐ I do not know

Of the following, which is NOT a zoonotic disease? (*mark all that apply*)

- ☐ Rabies
- ☐ Chickenpox
- ☐ Tapeworms
- ☐ Brucellosis
- ☐ Leptospirosis
- ☐ I do not know

How do you contract a zoonotic disease?

- ☐ From public transportation
- ☐ From spoiled food
- ☐ From unprotected sex with humans
- ☐ From toilet seats
- ☐ From animals
- ☐ I do not know

Of the following, which is/are NOT currently recognized forms of Hepatitis (*mark all that apply*)?

- ☐ Hepatitis A
- ☐ Hepatitis D
- ☐ Hepatitis F
- ☐ Hepatitis G
- ☐ Hepatitis Z
- ☐ I do not know

All types of Hepatitis primarily affect the:

- ☐ Liver
- ☐ Lungs
- ☐ Skin
- ☐ Heart
- ☐ Brain
- ☐ I do not know

Which of the following is the best way to PREVENT spreading infectious disease?

- ☐ Go out in public
- ☐ Avoid all animals
- ☐ Wash your hands frequently and thoroughly
- ☐ Avoid all people
- ☐ Wipe everything down with a cloth before you use

☐ **Yes**, I would like to receive the results of my dog's Hepatitis E screening. Please mail the information to me.

☐ **No**, I would not like to receive the results of my dog's Hepatitis E screening.

Thank you for your participation!

APPENDIX E: Canine Phlebotomy Sites

Blood draw from the forepaw

Blood Draw from neck