A STUDY OF POSTMORTEM SODIUM CONCENTRATIONS IN THE BRAIN, AQUEOUS HUMOR, AND CEREBROSPINAL FLUID OF HORSES RECEIVED AT OKLAHOMA ANIMAL DISEASE DIAGNOSTIC LABORATORY

By

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This project was initiated after a case of high sodium was found in brain tissue by the Oklahoma Animal Disease Diagnostic Laboratory and after numerous requests from veterinarians and diagnosticians for data on normal concentrations of brain sodium.

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NOMENCLATURE

AA	Atomic absorption spectrometer
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CC	Cubic centimeter
CNS	Central nervous system
CSF	Cerebrospinal fluid
ECF	Extracellular fluid
g	Gram
GLM	General linear model
ICF	Intracellular fluid
ICP-MS	Ion coupled plasma mass spectrometry
i.v.	Intravenous
L	Liter
lb	Pound
meq	milliequivalents
mL	Milliliter
mmol	millimole
Na+	Sodium ion
Nm	Nanometer
NSD	No significant difference
OADDL	Oklahoma Animal Disease Diagnostic Laboratory
ppm	Parts per million
q.s.	Sufficient quantity
RSD	Relative standard deviation
SD	Standard deviation

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CHAPTER I

INTRODUCTION

Background

Water deprivation/sodium ion toxicosis is a condition that is primarily observed in swine, cattle, and poultry. Salt poisoning, sodium ion toxicity, and hypernatremia are other names that are used synonymously for this condition. While sodium ion toxicity is more common in other species, this condition can affect horses. Brain, cerebrospinal fluid (CSF), and aqueous humor sodium concentrations are well documented in many species, but as yet no data has been published on the horse. These sodium concentrations are useful data for complete and accurate diagnosis of water deprivation/sodium ion toxicosis. There is a paucity of brain sodium data in animals other than cattle and swine but normal ranges are expected to be similar[1-5].

Sodium is an essential part of an animals' diet and body composition. An overview of sodium's role in the diet and body physiology is important and precedes the results and discussion.

Sodium and Water in the Diet

Sodium, an essential nutrient, is added to prepared feeds and offered in mineral supplements (loose or block form) to increase palatability or as an intake limiting agent when supplements are fed ad libitum. Most sodium needs can be met with 50-60 g of supplemental salt daily and horses often eat to meet their needs, rarely consuming enough

to result in toxicity as long as unrestricted fresh water is available[6, 7]. Consumption of salt is not usually an issue unless water is limited. Sodium should compose 0.1% to 0.5% of the ration depending on the use of the horse and other factors with the maximum tolerable level being around 3%[8]. High environmental temperatures and increased activity increase the requirement of salt in the diet due to loss in sweat [6]. The acute toxic dose of salt in swine, ruminants and horses is reported to be 2.2 g/kg [2, 5, 9].

Clean water should be provided at all times in ample amounts. It is estimated that a 1000 lb horse can consume 4-15 gallons of water per day depending on activity levels[6]. High environmental temperatures, increased activity, lactating mares, and mares in the last 1/3 of gestation all need larger quantities of water [6]. Water consumption also increases with high roughage, high dry matter intake diets to prevent impaction in the intestine [8]. A recommendation for acceptable salinity levels in water is complex due to a variety of salts present in the water. It is recommended that the concentration of total salt be less than 0.9 % for horses and preferably no more than 0.35 %[10].

Direct versus Indirect Sodium Toxicosis

Excessive sodium intake in the form of feed or water causes direct sodium toxicosis[4]. Direct sodium toxicosis can also occur from ingestion of salt water from oil field sites. Salt water is most often consumed during winter months when fresh water becomes frozen and there is no other available alternative. Salt consumed dissolved in water is more harmful than salt consumed in dry feed since the animal cannot consume additional water to dilute the sodium intake[2].

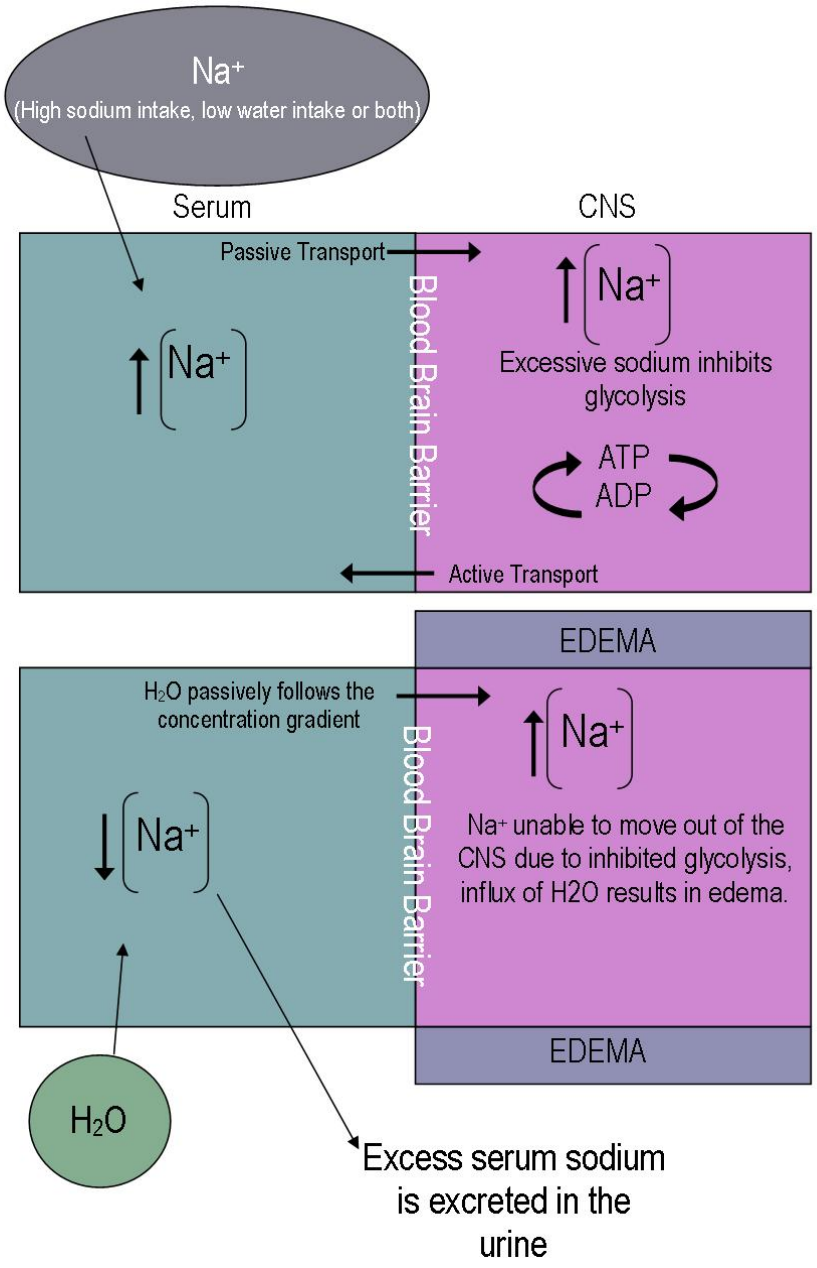
Indirect sodium toxicosis is generally caused from restricted water intake and progresses over 4-7 days [4]. Water deprivation can occur from several factors including

frozen water, inability to find water due to new surroundings, failure of mechanical watering devices, overcrowding, and unpalatable water.

Mechanism of Action of Water deprivation/Sodium Ion Toxicity

Sodium is rapidly absorbed from the GI tract and causes an increase in serum sodium concentrations[8]. When dehydration occurs, sodium passively diffuses across the blood brain barrier increasing the concentration of sodium in the CSF[2, 6, 8, 10, 11]. To maintain this osmotic gradient created by the dehydration, brain cells also increase their concentrations of sodium to prevent loss of water resulting in cell shrinkage[11]. This increase of sodium in the cells inhibits anaerobic glycolysis resulting in a decrease in available energy (ATP) in the cell[2, 6, 8, 10, 11]. This loss of energy prevents sodium from being actively transported out of the cell[2, 6, 10, 11]. When water is reintroduced, serum concentrations of sodium return to normal and excess sodium is excreted in the urine[2, 6, 8]. However, sodium is still trapped in the cells of the CSF and brain due to the inhibited anaerobic glycolysis. The lack of energy still prevents this sodium from being actively pumped out of the cell[6, 10]. Water passively diffuses into the cells of the CNS following this osmotic gradient of high sodium in the cell and low sodium outside the cell[2, 6, 8, 11]. This influx of water into the cell causes cells to swell resulting in edema[2, 10]. Edema in the brain causes the clinical signs seen[2, 6, 8, 10, 11].

Figure 1: Mechanism of Action



Clinical Signs of Water Deprivation/Sodium Ion Toxicity

Animals with water deprivation/Na+ toxicity can exhibit mild clinical signs before they begin the classic neurologic signs associated with the disorder. These clinical signs may often be overlooked.

With the initial onset of water deprivation in swine, thirst, restlessness, vomiting, diarrhea, and pruritis are the first signs that can be noted [2, 4, 12]. As signs progress, usually 1-5 days after initial water deprivation, swine develop largely neurologic signs, including aimless wandering, blindness, circling, pivoting on one foot, dog sitting, head pressing, and seizures with opisthotonos[2, 12].

With cattle, the early signs often differ. When excessive salt intake is the cause, weakness and severe gastroenteritis lead to dehydration and death within 24 hours [2]. Free access to water after a period of deprivation or after an excessive salt intake leads to the typical neurologic signs. These signs include blindness, circling, incoordination, seizure, head pressing, fine muscle twitching, aggressiveness to get to water, belligerence, nasal discharge, and slobbering [2, 4, 13]. A common sequela of water deprivation/sodium ion toxicosis is dragging of the hind feet or knuckling at the fetlock [2, 4, 12, 13].

Less is known about the clinical signs other species exhibit. Poultry show signs of thirst, dyspnea, wet droppings, fluid discharge from the beak, and weakness or paralysis of the legs [2, 4, 13]. Dogs, although rarely affected, generally only show signs of gastrointestinal upset such as vomiting and diarrhea with water deprivation/sodium ion toxicity. Horses are expected to show similar clinical neurologic signs as those in cattle but, to date, no studies have been done to confirm this.

Differential Diagnosis of Water Deprivation/Sodium Ion Toxicosis

There is a large list of other conditions to consider when a horse presents with the clinical signs mentioned above. The chart below is by no means a complete list and does not include conditions like trauma, tumors, all toxins and genetic disorders.

Viruses	Bacteria	Parasites	Toxins	Toxic Plants
Eastern Equine Encephalitis	Bacterial Meningitis	Equine Protozoal Myelitis	Lead	Locoweed
Western Equine Encephalitis	Brain Abscess	Visceral larval migran	Fumonisin	Senecio
Venezuelan Equine Encephalitis	Osteomyelitis	Cryptococcus	Botulism	Yellow Star Thistle
Equine Herpes Virus-1			Organophosphates	Brackenfern
West Nile Virus				Horsetail
Rabies				Sudan Grass
				Crotalaria
				Russian Knapweed

Diagnosing Water Deprivation/Sodium Ion Toxicosis

When diagnosing water deprivation/sodium ion toxicity a thorough history is very important to obtain. This is often difficult to do as the caretaker is often unwilling to admit poor husbandry practices that lead to restricted water intake. In addition to history, diagnosis made through clinical signs, post mortem histopathology examination of brain tissue, and post mortem toxicology testing of brain tissue for sodium concentrations. A combination of these, rather than relying on only one, is the best and most accurate way

to diagnose water deprivation/sodium ion toxicosis. Here normal brain sodium concentrations are established, it can be the sole factor confirming a diagnosis.

Serum and CSF can be collected from live animals and analyzed for sodium concentrations. Normal serum sodium concentrations range from 3150 - 3288 ppm in the horse [11]. Normal CSF sodium concentrations are between 3219 – 3448 ppm in the horse [14]. Although aqueous humor has been collected on live animals in a research setting, this is unlikely to become a routine clinical procedure. In most livestock species, serum sodium concentrations greater than 3678 ppm would indicate hypernatremia [13]. If serum sodium concentrations are within normal limits and water deprivation/sodium ion toxicosis is suspected, it is best to do additional testing on the CSF for sodium concentrations[2, 9, 12]. The sodium concentrations found in CSF remain elevated for a longer duration than serum sodium concentrations, which tend to quickly decline after access to water, either oral or intravenously.

Gross lesions are few and nonspecific in cases of water deprivation or sodium ion toxicity. Gastric contents can appear abnormally dry and the mucosa may show some degree of irritation together with ulceration and hemorrhage[1]. Grossly, there may be cerebral swelling evidenced by the coning of the cerebellum and flattening of cerebral gyri and bulging of cut surfaces (figures 2 & 3).

Figure 2: Bulging at the cut surface on a bovine brain. Photo courtesy of OSU Pathology Department.

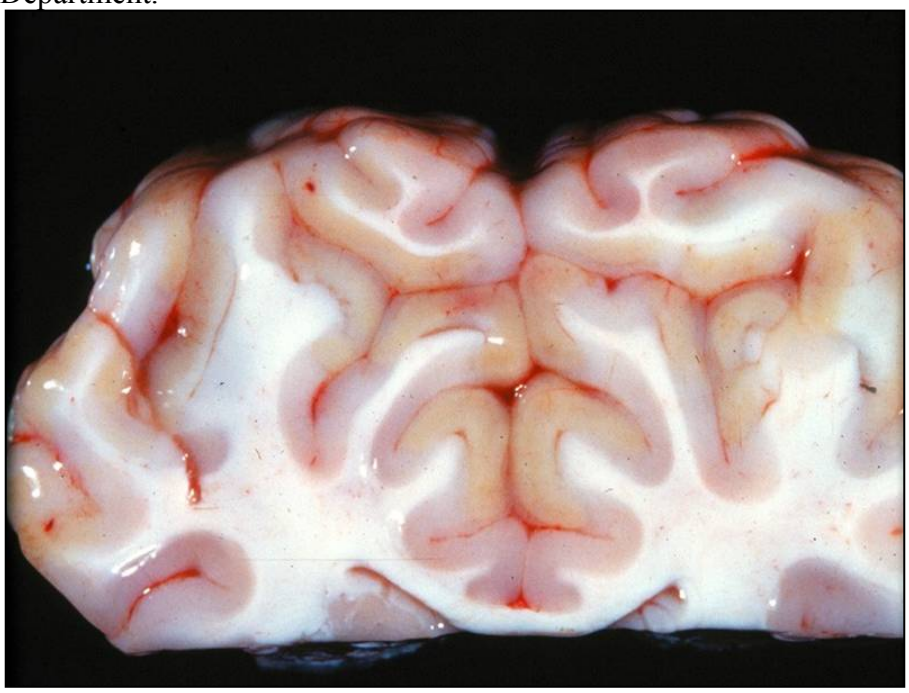
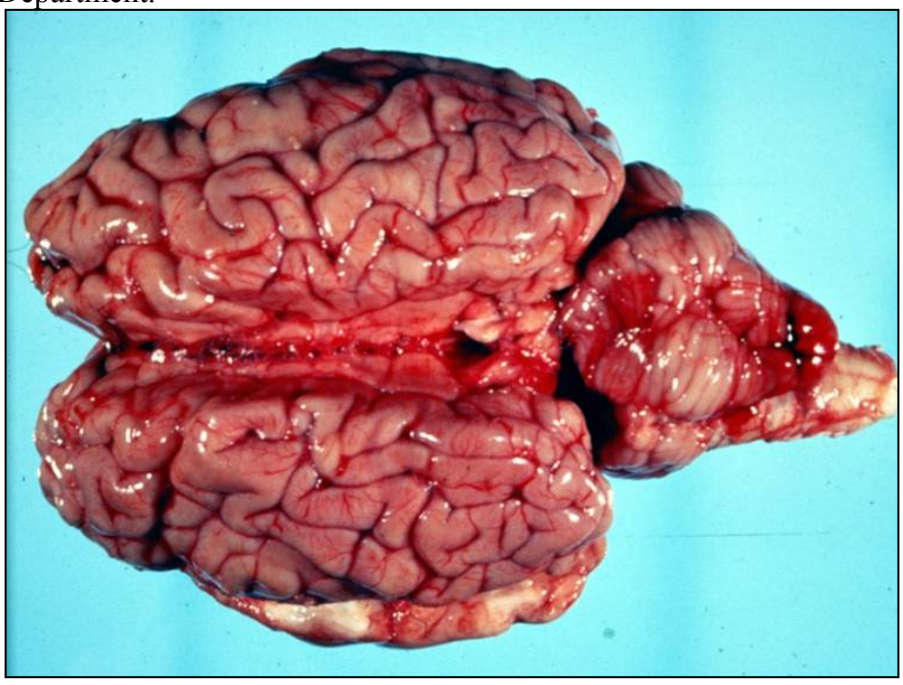
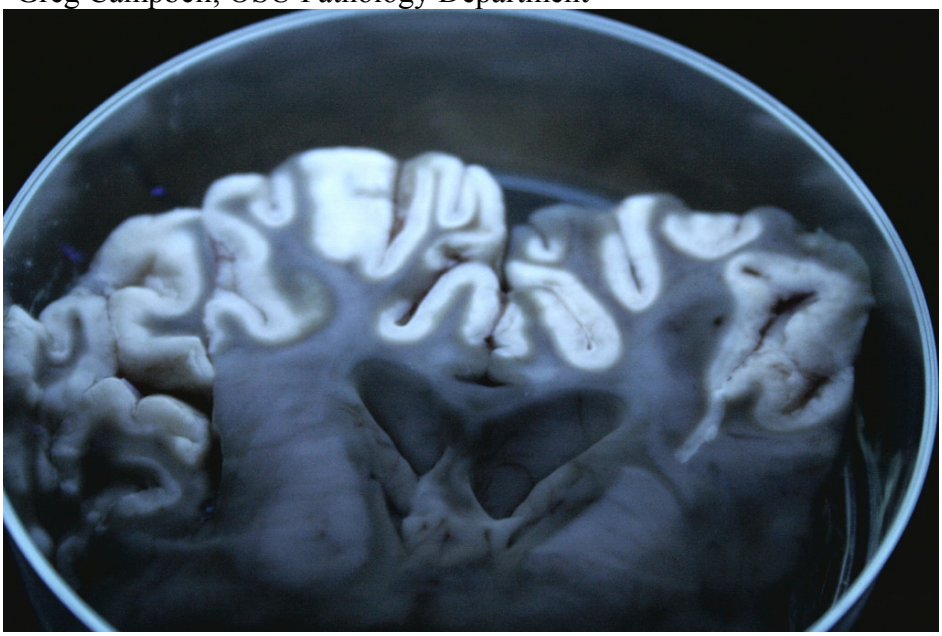


Figure 3: Cerebellar Coning on a bovine brain. Photo courtesy of OSU Pathology Department.



In cattle, the cut surface of the brain is examined under a Wood's lamp, fluorescing of the collapsing grey matter can be seen indicating neuronal degradation (figure 4). This is more often seen in polio cases than with water deprivation or lead toxicity.

Figure 4: Fluorescing of collapsing grey matter on a bovine brain. Photo courtesy of Dr. Greg Campbell, OSU Pathology Department



Brain tissue, aqueous humor, and cerebrospinal fluid are specimens that can be harvested post mortem for determining sodium concentrations. There is a paucity of literature that could be found for normal brain sodium concentrations in horses. Some references differ greatly in their values of normal versus toxic, with overlap occurring between studies

Adequate brain sodium concentrations in cattle can range from 800-1400 ppm and toxic ranges from 1800 – 1400 ppm (see Table 1)[3, 15]. Normal aqueous humor in cattle ranges from 2990-3450 ppm, serum 3140-3450 ppm and CSF 3036-3266 ppm [3, 4].

Contradictory values for brain sodium concentrations are found in literature. For example, in swine brain sodium concentrations of 1850-2030 are considered adequate but 1500-1950 ppm are classified as toxic [3, 16]. Normal aqueous humor sodium concentrations in swine range from 3220-3335 ppm, serum range from 3220-3450 ppm and CSF range from 2990-3450 ppm [3, 16]. One study finds a poor relationship between ocular fluid and serum concentrations of electrolytes in swine [17].

Normal serum sodium concentrations in the horse are reported to range from 132 mEq/L to 146 mEq/L [18, 19, 20, 21, 22]. Normal aqueous humor concentrations in the horse are reported to be 128 mEq/L to 140 mEq/L[19, 23]. One text reports antemortem sodium concentrations in aqueous humor to be 117.4 mEq/L [24]. No reported brain sodium concentrations in horses could be found through multiple literature searches using PubMed as well as other literature search engines.

Table 1: Normal sodium concentrations (ppm)

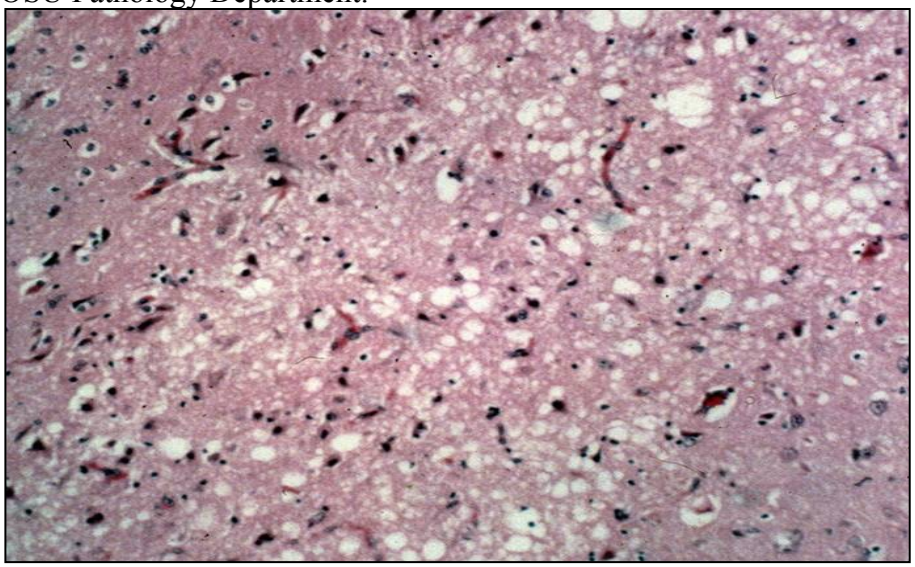
Reference	Brain (ppm)			CSF (mEq/L)			Aqueous Humor (mEq/L)			Serum (mEq/L)		
	Cattle	Swine	Horse	Cattle	Swine	Horse	Cattle	Swine	Horse	Cattle	Swine	Horse
Puls[3]	800-1400	1850-2030		132-142	130-150		130-150	140-145		135-150	140-150	
Martin[15]	1025	1800										
Straw[16]					140-145						140-145	
Radostits[27]											135-145	
Smith[9]												
Reed[20]												
Plumlee[4]	1600	1800		135-155			129-156			135-155		
Gupta[1]	1600	1800		135-150						132-152	135-150	132-146
Osweiler[13]				135-150	135-150					135-155	135-155	
Robinson[28]						140-150						134-143
Beasley[2]				135-155						135-155		
Osweiler[34]				130-140	130-140					135-145	135-145	
Parton[7]										135-150	140-150	
McLaughlin[23]									128-140			133-147
Osweiler[19]							132-156					
Mayhew[25]						140-150						
Gelatt[24]									117.4			

Table 2: Toxic sodium concentrations (ppm)

Reference	Brain (ppm)			CSF (ppm)			Aqueous Humor (mEq/L)			Serum (mEq/L)		
	Cattle	Swine	Horses	Cattle	Swine	Horses	Cattle	Swine	Horses	Cattle	Swine	Horses
Puls[3]	1800-2400	1500-1950		160-211	160-210		>180			150-250	180-200	
Martin[15]	1800											
Straw[16]		>1800			>160						>160	
Radostits[27]	2230-4250						172-218				170-210	
Smith[9]	>1800			>160						>160		
Reed[20]												
Plumlee[4]	>2000						172-218					
Gupta[1]	>2000	>2000										
Murphy[5]	>1800	>1800		>145	>145							
Osweiler[13]	>2000	>2000		>160	>160					>160	>160	
Rhoder[26]	>1800	>1800		>180	>180					>180	>180	
Robinson[11]												
Howard[12]	>1800	>1800								>160	>160	
Beasley[2]	>1800	>1800		>160	>160					>160	>160	
Osweiler[34]	>2000	>2000		>160	>160					>160	>160	
Parton[7]										>200	180-200	
Osweiler[19]	>1620						172-218			>200		

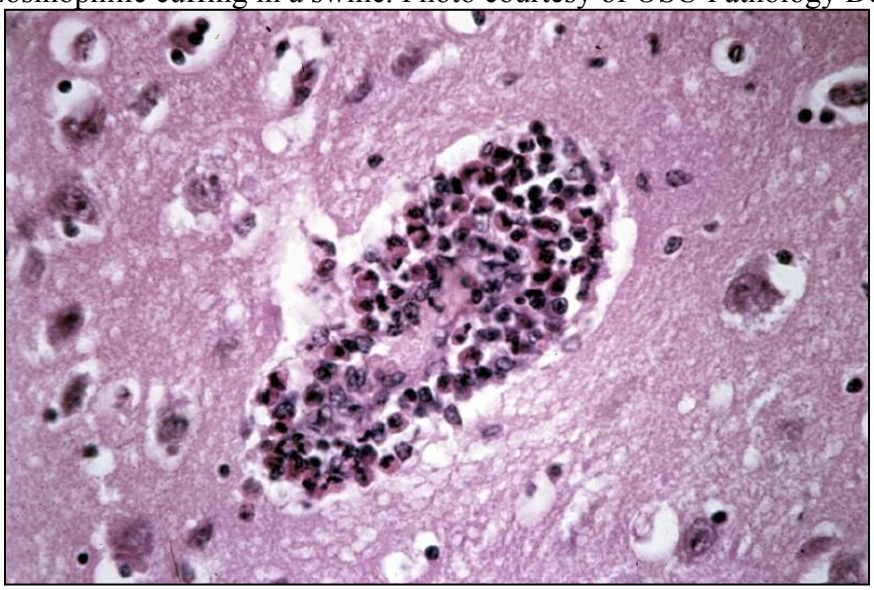
In all veterinary species, microscopic brain lesions can be mild or non-existent with water deprivation/sodium toxicosis. When examining tissues, histological differences can be seen between cattle and swine. Cattle will have polioencephalomalacia with neuronal degradation[15] (figure 5).

Figure 5: Histopathology lesion Polioencephalomalacia in a bovine. Photo courtesy of OSU Pathology Department.



This microscopic lesion overlaps with sulfur toxicosis, lead toxicosis, ischemia and hypoglycemia. Swine show a lesion unique to their species. Eosinophilic cuffing is characteristic of water deprivation/sodium ion toxicosis if death occurs within the first 48 hours [2, 13](figure 6). After the first 48 hours, mononuclear cells begin to replace the eosinophills[1, 2, 4].

Figure 6: Eosinophilic cuffing in a swine. Photo courtesy of OSU Pathology Department.



Treatment of Water Deprivation/Sodium Ion Toxicosis

Treatment of water deprivation/sodium ion toxicity is difficult and carries at best a guarded prognosis. Approximately 50% of affected animals have fatal outcomes due to the difficult task of returning the animal to normal water and electrolyte balance [1, 13]. Animals must be given small amounts of water frequently over a 2-3 day period. By limiting the water intake, serum sodium concentrations are slowly returned to normal, preventing large amounts of water from following the concentration gradient into the brain and causing cerebral edema. It is recommended that intake be limited to 0.5% of the body weight every 60 minutes until the animal is re-hydrated [4].

Mannitol 25% can be given i.v. to treat cerebral edema but is contraindicated if brain hemorrhage is suspected. Mannitol decreases blood viscosity, increases blood osmolality and causes cerebral vasoconstriction which facilitates a decrease in cerebral edema [29]. This treatment can be repeated every 4-6 hours up to 24 hours. Other drug

therapies that will reduce brain swelling include dimethly sulfoxide (DMSO), dexamethasone, and glycerin. Diazepam can be given to control seizures [4].

Objective

While water deprivation/sodium ion toxicosis is not commonly thought of as a condition affecting horses, horses suspected of being deprived of water have been submitted to Oklahoma Animal Disease Diagnostic Laboratory for post mortem examination. The results of these exams have been inconclusive, in part because criteria for diagnosis in horses are not established.

The primary objective of this study was to establish a range of post mortem concentrations of sodium in the brain of a population of horses submitted to OADDL without a clinical history suggestive of water deprivation/sodium ion toxicity. The opportunity was also used to examine postmortem aqueous humor and cerebrospinal fluid sodium concentrations

CHAPTER II

METHODOLOGY

Brain, cerebrospinal fluid and aqueous humor samples were collected from horses submitted to Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for post mortem examination from August 2003 thru January 2005. Samples were not harvested from horses with a history of neurologic disease. Due to varying condition of the animals submitted, brain, aqueous humor and CSF were not always available for harvest from each animal. Decomposition, predator removal and use of the specimen by pathologist or other laboratory sections for diagnostic testing determined the availability of samples.

Originally, 133 aqueous humor, 140 CSF, 122 frontal lobe and 121 cerebellum specimens were collected from 150 horses. Horses were then divided into various groups that were thought to have factors that could affect sodium values [30, 31]. The "total population" includes 150 horses.

- **Category**: Age
    - **Subcategories**: 0-1, 2-9, 10-19 or ≥20
- **Category**: Sex
    - **Subcategories**: Male or female
- **Category**: Breed
    - **Subcategories**: Quarter horse, thoroughbred and "other breeds"

- **Category**: Cause of death
    - **Subcategories**: Colic, infection, trauma or "other"
- **Category**: Use of euthanasia solution
    - **Subcategories**: Yes, no or unknown
- **Category**: Receiving fluids prior to death
    - **Subcategories**: Yes or no
- **Category**: Location
    - **Subcategories**: Oklahoma or "other states"
- **Category**: Type of animal
    - **Subcategories**: Racehorse or non-racehorse

Racehorses, horses that received fluids (colic, surgery), and horses with no history were excluded from the "total population". All others were included in the "standard population". The total number of specimens was reduced to 39 aqueous humor specimens, 39 CSF specimens, 42 frontal lobe specimens, and 43 cerebellum specimens. The "excluded population" were the animals that received fluids (colic or surgery), were racehorses, or had no reported case history. The results of the "total population", the "standard population", and "excluded population" are shown in Tables 7, 8 and 9.

Of the horses included in the "standard population", a comparison was made between horses that were necropsied within 1 day of death and within 2 days of death (table 10). No animals were necropsied that had been dead greater than 2 days due to autolysis. Generally animals are not submitted for necropsies that have been dead for an extended period of time. Racehorses, horses that received fluids (colic, surgery), and horses with no history were excluded from the standard population.

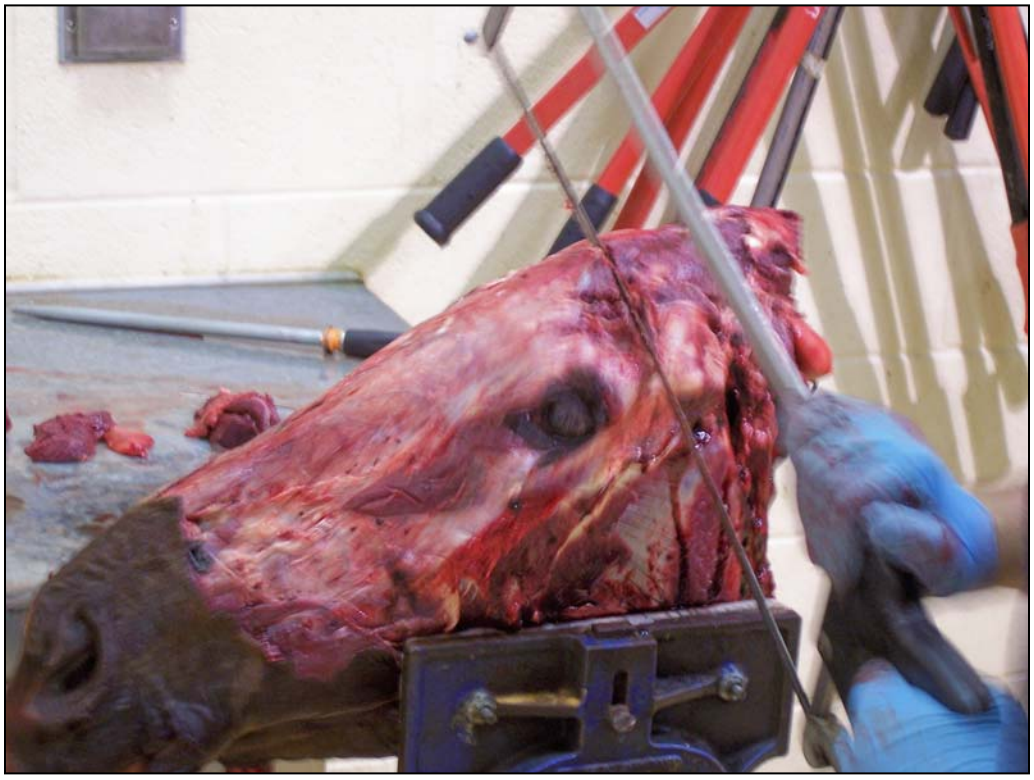
Ideally, a complete set of specimens would have been collected from each animal. Because these animals were submitted to OADDL for diagnosis, decomposition and use for diagnostic testing affected the availability of all specimens. Hemorrhage into CSF and overall difficulty collecting sufficient volume affected the number of CSF samples collected. Eyes were occasionally absent from the animal submitted due to removal by predators. Use of aqueous humor for electrolyte testing often depleted the specimen.

All samples collected were frozen until the time of processing. Preparation of tissue and fluid samples and sodium analysis done according to OADDL toxicology SOP TOX-AA-006.01[32]. This procedure is commonly used in accredited veterinary diagnostic laboratories that use atomic absorption techniques.

Harvesting and Preparation of Brain Tissue Samples

Brains were harvested according to the OADDL necropsy SOP NEC-PR-003.01[33] using a hacksaw making the first 2 cuts caudal on the skull starting at the inner margins of the occipital condyles and angling out. A third cut was made at the front of the cranial vault along a line approximately 2cm caudal to the lateral margin of the eye socket. Final cuts were made along the sides of the skull cap connecting the first and second cuts.

Figure 7: Removal of brain



A wedge was inserted into the cuts, and a prying motion reflected the skull cap allowing the brain to be removed. Samples were thawed and processed in small batches due to limited space and stored until testing.

Preparation of tissue samples began by harvesting 1 gram samples of tissue, +/- 0.1 grams, from the frontal lobe and 1 gram samples, +/- 0.1 grams, of tissue from the

cerebellum (figure 8 & 9). They were placed in individual acid washed Erlenmeyer flasks.

Figure 8: Equine Brain, cranial view

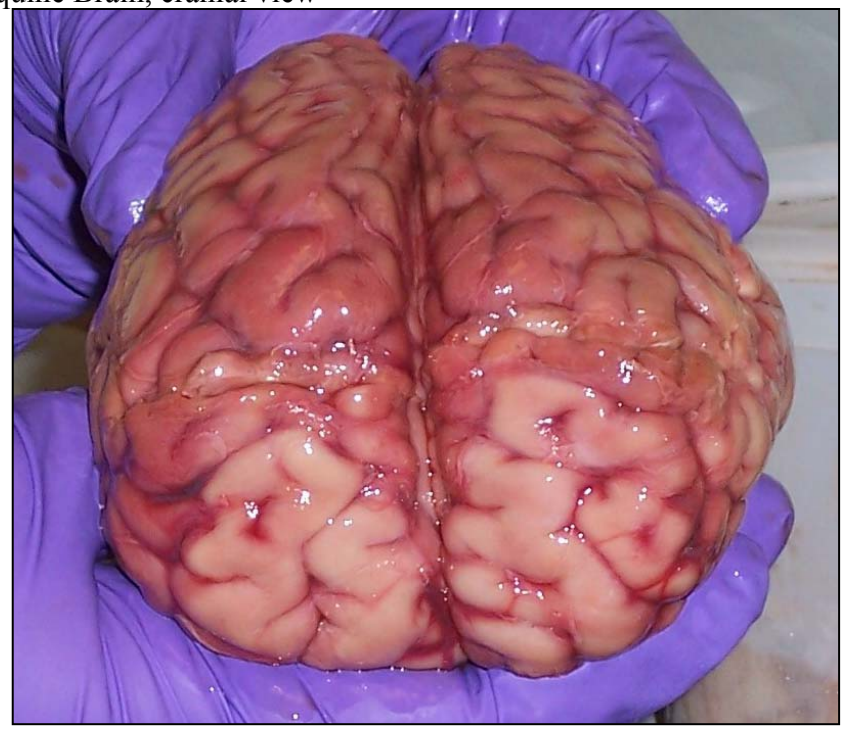


Figure 9: Equine brain, dorsal view

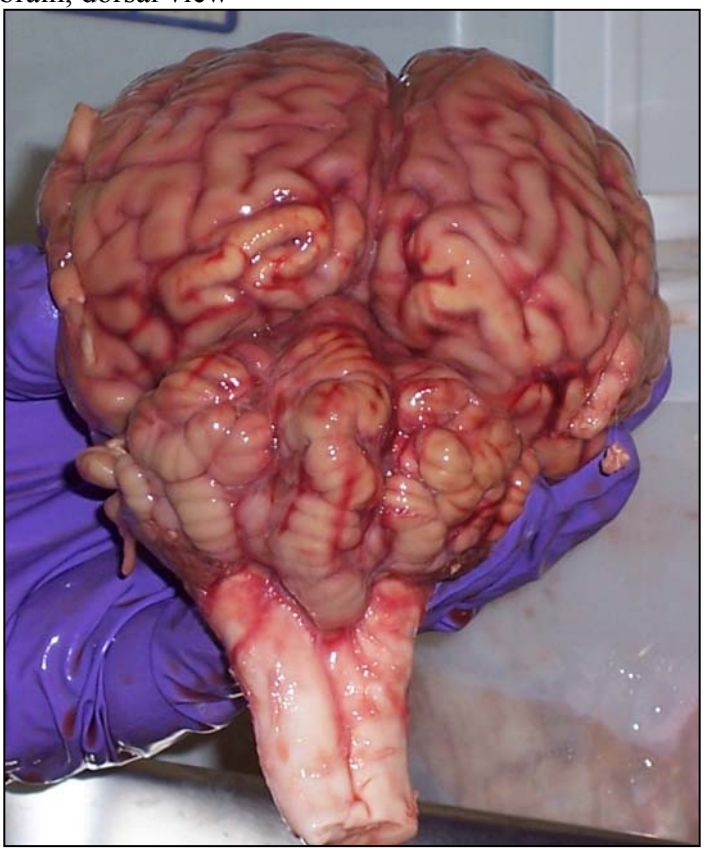


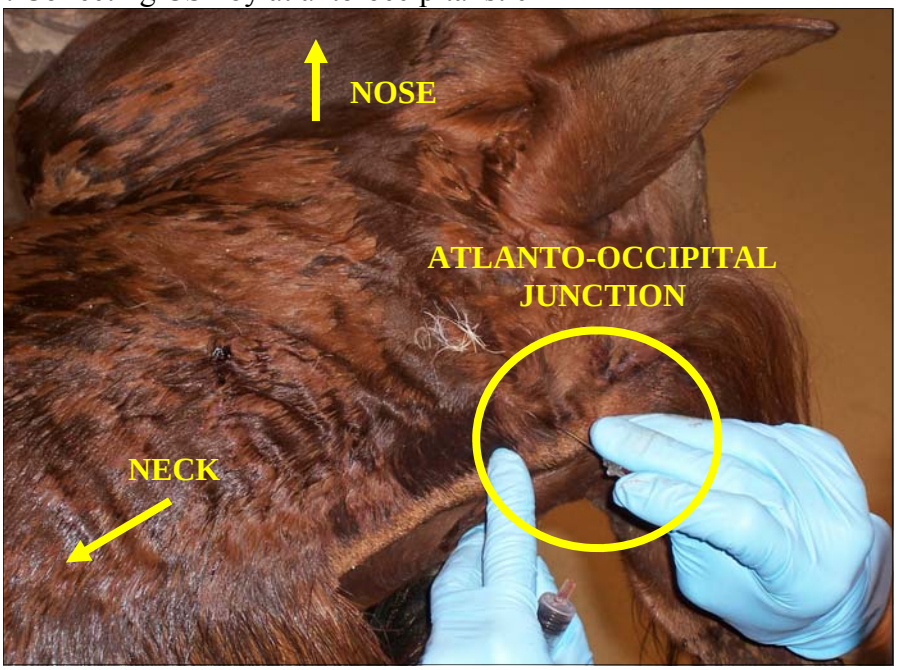
Figure 10: Samples digesting in Erlenmeyer flasks

Tissues were weighed using a Mettler-Toledo 620-s electronic balance and labeled with sample type, weight and identification number. Ten milliliters of a 50% nitric acid/ 50% distilled deionized water solution were then added to the flask and warmed on a hotplate until the volume had reduced to approximately 3 mL of digest (figure 10). . The sample was weighed and the final volume was brought up to 1L in a volumetric flask. Digests were filtered using Whattman paper filter #4 into acid washed volumetric flasks. The flask was rinsed multiple times with distilled water and poured through the filter to ensure the total digested brain sample was in the final dilution. Samples were then q.s. to 1L final volume with distilled deionized water in volumetric flasks and mixed. Aliquots of 125mL were placed in polyethylene bottles labeled with sample weight and case number and stored at room temperature until all samples were processed and analysis could be performed.

Harvesting and Preparation of Cerebrospinal Fluid and Aqueous humor

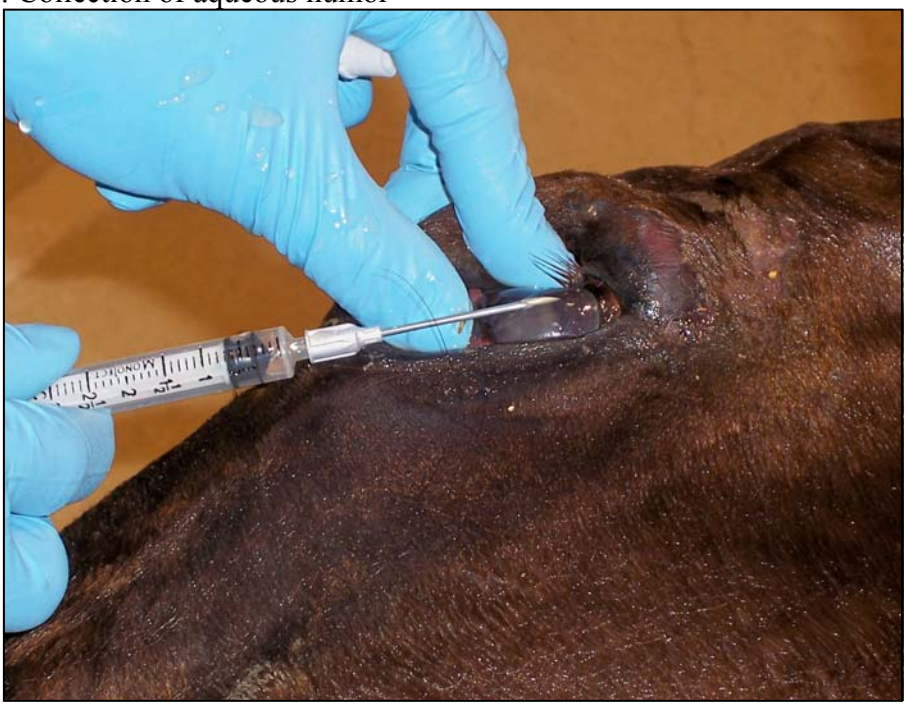
Cerebrospinal fluid was harvested with a syringe and an 18 gauge spinal needle at the atlanto-occipital junction (figure 11).

Figure 11: Collecting CSF by atlanto-occipital stick



Aqueous humor was collected with a syringe and 18 gauge needle by inserting the needle through the corneas to collect the aqueous humor (figure 12). All samples were frozen at 0 C° until all sample collection was completed.

Figure 12: Collection of aqueous humor

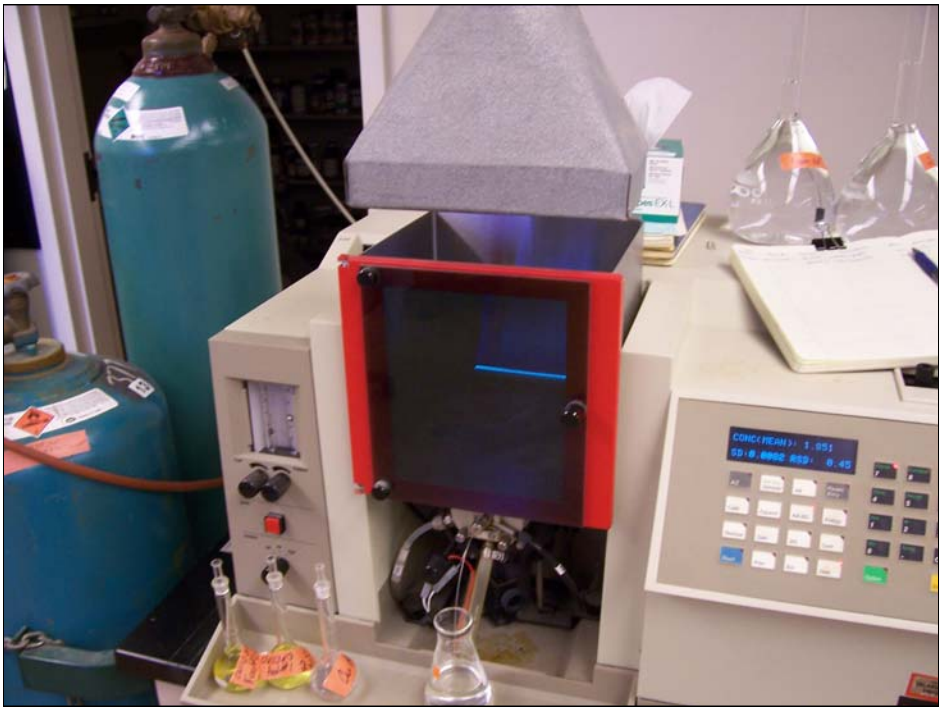


Methods for preparation of CSF and aqueous humor samples were identical. One milliliter of CSF or aqueous humor was pipetted into an acid washed volumetric flask and q.s. to 1L using distilled deionized water and mixed. Small aliquots of 125mL were placed in polyethylene bottles labeled with sample type, sample volume and case number and stored at room temperature until time of analysis.

Analysis of Samples

Samples were analyzed in 2 batches on a Perkin Elmer 3110 flame furnace atomic absorption spectrometer (figure 13).

Figure 13: Perkin Elmer 3110 flame furnace



The AA flame furnace lamp was set to a wave length of 589nm and the furnace slit was set to 0.4nm. Sodium standards at concentrations of 1ppm, 2ppm, and 5ppm were used to calibrate the AA and the response was linear. Samples and standards were read using an air/acetylene flame. Three replicates were performed and recorded for each sample and the RSD was within acceptable limits for diagnostic purposes of less than 2

RSD, with the majority being less than 1. The average of the 3 replicates was the value used in the statistical analysis and all fell within the standard curve.

Atomic absorption uses light to measure gas-phase concentration of atoms. Each element absorbs a specific wavelength of light which is emitted from a hollow cathode lamp. As the sample is vaporized by the flame, light is passed through the flame and, elements in the sample are excited to a higher energy state. They are excited by the light shining through the flame path not by the flame itself. The increased excitation of the element in question causes less light to pass through to the detector. A monochromator disperses the light and the detector reads the amount of reduction in light due to the absorption of light by the element in question. The amount of light identified by the detector can be correlated back to the quantity of analyte the digest contains.

Atomic absorption is able to detect below 1 part per million and is a widely used method for determining concentration of metals and nonmetals in a specimen. Ion coupled plasma mass spectrometry (ICP-MS) is an increasingly more common means for determining metal and nonmetal concentrations in a specimen. ICP-MS does have a lower detection limit than AA, with the ability to detect on the ppb level. Another advantage with ICP-MS is the ability to analyze multiple metals and nonmetals at one time. However, atomic absorption is a much more affordable means of testing specimens than ICP-MS due to the high equipment cost, high maintenance cost, and technician expertise required to operate an ICP-MS.

Statistical Analysis

The sodium analysis performed on the total population of samples was originally divided into the categories and subcategories previously mentioned and analyzed using a

t-test, general linear model and ANOVA procedures, as shown on tables 3-6. These procedures include variable analysis by group, and variability within group.

The normal range of sodium in equine frontal lobe was calculated using a 95% confidence interval by adding or subtracting two standard deviations from the "standard population" mean [30]. Values that fell outside this 95% confidence interval were examined using a statistically robust rule-of-thumb for examining outliers of a population[31]. If the distance between the 2 highest values is determined to be greater than one third of the range of all values, the highest value would be considered an "outlier" of the population[31]. This system can be applied to the second highest to determine if it is an outlier.

CHAPTER III

RESULTS

The hypothesis for the results on tables 3-6 was that there might be significant differences in the sodium values particularly in animals that received fluids.

There were 150 horses in the total population. Of those, 37 were thoroughbreds, 73 quarter horses and 40 other breeds. Fifty two of the population were racehorses and 98 were not racehorses. Of the 52 racehorses, 33 were thoroughbreds which were 63% of the population, 11 were quarter horses which were 21% of the population and 8 were other breeds making up the15% of the population. Ninety eight of the total population were not racehorses. Of the 98, 4 were thoroughbreds which were 4% of the population of non-racehorses, 62 were quarter horses which were 63% of the population and 32 were other breeds making up the final 33% of the population.

Table 3: Total Population, frontal lobe mean for each subcategory, p-value, and decision.

Variable	Group	Mean Value (ppm)	P Value	Significant Difference
Gender	Male (n=97)	1327	0.86	No
	Female (n=53)	1318		
Area	Oklahoma (n=102)	1325	0.96	No
	Other States (n=48)	1328		
Fluids	Received fluids (n=42)	1295	0.34	No
	Did not receive fluids (n=108)	1340		
Racehorse	Yes (n=52)	1325	0.9	No
	No (n=98)	1319		
Age	0-1 yrs (n=49)	1289	0.63	No
	2-9 yrs (n=78)	1344		
	10-19 yrs (n=15)	1343		
	≥20 yrs (n=8)	1230		
Cause of Death	Colic (n=26)	1345	0.85	No
	Infection (n=9)	1383		
	Trauma (n=52)	1311		
	Other (n=42)	1304		
	Unknown (n=21)	1360		
Euthanized	Yes (n=95)	1303	0.25	No
	No (n=49)	1378		
	Unknown (n=6)	1277		
Breed	Quarter Horse (n=73)	1293	0.34	No
	Thoroughbred (n=37)	1348		
	Other (n=40)	1363		

Table 4: Total Population, cerebellum mean for each subcategory, p-value, and decision.

Variable	Group	Mean Value (ppm)	P Value	Significant Difference
Gender	Male (n=97)	1390	0.86	No
	Female (n=53)	1402		
Area	Oklahoma (n=102)	1422	0.95	No
	Other States (n=48)	1414		
Fluids	Received fluids (n=42)	1376	0.38	No
	Did not receive fluids (n=108)	1439		
Racehorse	Yes (n=52)	1418	0.62	No
	No (n=98)	1389		
Age	0-1 yrs (n=49)	1458	0.83	No
	2-9 yrs (n=78)	1418		
	10-19 yrs (n=15)	1367		
	≥20 yrs (n=8)	1544		
Cause of Death	Colic (n=26)	1504	0.37	No
	Infection (n=9)	1489		
	Trauma (n=52)	1344		
	Other (n=42)	1402		
	Unknown (n=21)	1600		
Euthanized	Yes (n=95)	1397	0.72	No
	No (n=49)	1466		
	Unknown (n=6)	1390		
Breed	Quarter Horse (n=73)	1364	0.26	No
	Thoroughbred (n=37)	1408		
	Other (n=40)	1518		

Table 5: Total Population, aqueous humor mean for each subcategory, p-value, and decision.

Variable	Group	Mean Value (ppm)	P Value	Significant Difference
Gender	Male (n=97)	3341	0.29	No
	Female (n=53)	3177		
Area	Oklahoma (n=102)	3223	0.18	No
	Other States (n=48)	3456		
Fluids	Received fluids (n=42)	3097	0.05	No
	Did not receive fluids (n=108)	3385		
Racehorse	Yes (n=52)	3534	0.06	No
	No (n=98)	3170		
Age	0-1 yrs (n=49)	3189	0.71	No
	2-9 yrs (n=78)	3386		
	10-19 yrs (n=15)	3217		
	≥20 yrs (n=8)	3198		
Cause of Death	Colic (n=26)	3273	0.99	No
	Infection (n=9)	3148		
	Trauma (n=52)	3303		
	Other (n=42)	3297		
	Unknown (n=21)	3387		
Euthanized	Yes (n=95)	3281	0.89	No
	No (n=49)	3276		
	Unknown (n=6)	3475		
Breed	Quarter Horse (n=73) [A,B]	3260	0.01	Yes
	Thoroughbred (n=37) [A]	3686		
	Other (n=40) [B]	3038		

Table 6: Total Population, CSF mean for each subcategory, p-value, and decision.

Variable	Group	Mean Value (ppm)	P Value	Significant Difference
Gender	Male (n=97)	3152	0.3526	No
	Female (n=53)	3487		
Area	Oklahoma (n=102)	3258	0.9287	No
	Other States (n=48)	3228		
Fluids	Received fluids (n=42)	2941	0.0753	No
	Did not receive fluids (n=108)	3412		
Racehorse	Yes (n=52)	3476	0.3046	No
	No (n=98)	3125		
Age	0-1 yrs (n=49)	3095	0.8365	No
	2-9 yrs (n=78)	3272		
	10-19 yrs (n=15)	3387		
	≥20 yrs (n=8)	2707		
Cause of Death	Colic (n=26)	2876	0.6929	No
	Infection (n=9)	3358		
	Trauma (n=52)	3260		
	Other (n=42)	3493		
	Unknown (n=21)	2851		
Euthanized	Yes (n=95)	3263	0.7945	No
	No (n=49)	3290		
	Unknown (n=6)	3750		
Breed	Quarter Horse (n=73) [A, B]	3148	0.0134	Yes
	Thoroughbred (n=37) [A]	3926		
	Other (n=40) [B]	2769		

Table 7: Mean, standard deviation, and range for standard population

Specimen	Mean	Standard Deviation	Range	95% Confidence Interval
Frontal Lobe n=43	1336 ppm	194 ppm	1039 ppm - 2090 ppm	948 ppm - 1724 ppm
Cerebellum n=42	1395 ppm	284 ppm	915 ppm - 2552 ppm	827 ppm - 1963 ppm
Aqueous Humor n=39	3147 ppm	718 ppm	2014 ppm - 6129 ppm	1711 ppm - 4583 ppm
CSF n=39	3143 ppm	1313 ppm	1992 ppm - 9176 ppm	1992 ppm - 9697 ppm

Table 8: Mean, standard deviation, and range for total population

Specimen	Mean	Standard Deviation	Range	95% Confidence Interval
Frontal Lobe n= 122	1326 ppm	234 ppm	890 ppm - 2453 ppm	714 ppm - 1958 ppm
Cerebellum n=121	1419 ppm	436 ppm	636 ppm - 4933 ppm	391 ppm - 2399 ppm
Aqueous Humor n= 133	3288 ppm	871 ppm	2014 ppm - 8677 ppm	840 ppm - 5454 ppm
CSF n=140	3250 ppm	1506 ppm	1992 ppm - 9697 ppm	486 ppm - 11203 ppm

Table 9: Mean, standard deviation, and range for excluded population

Specimen	Mean	Standard Deviation	Range	95% Confidence Interval
Frontal Lobe n=79	1338 ppm	264 ppm	890 ppm - 2453 ppm	810 ppm - 1886 ppm
Cerebellum n=79	1442 ppm	510 ppm	636 ppm - 4933 ppm	422 ppm - 2884 ppm
Aqueous Humor n=94	3296 ppm	918 ppm	2014 ppm - 8677 ppm	1460 ppm - 5132 ppm
CSF n=101	3171 ppm	1326 ppm	1992 ppm - 9697 ppm	519 ppm - 5823 ppm

Table 10: Mean, standard deviation, and range for animals necropsied on day 1 vs. day 2

Sample	Mean	Standard Deviation	Range	95% Confidence Interval
Frontal Lobe day 1 n=23 day 2 n=16	1296 ppm 1299 ppm	151 ppm 149ppm	1039 - 1764 ppm 1196 - 1810 ppm	994 - 1598 ppm/ 1001 – 1597 ppm
Cerebellum day 1 n=23 day 2 n=16	1375 ppm 1377 ppm	249 ppm 248ppm	976 - 1875 ppm 915 - 2106 ppm	877 - 1873 ppm/ 881 - 1873 ppm
Aqueous Humor day 1 n=24 day 2 n=19	3432 /3436 ppm	1013/1012ppm	2528-4996/2090-6129 ppm	1406- 5458/ 1412- 5460ppm
CSF day 1 n=25 day 2 n=17	3358/3322ppm	1688/1586ppm	2355-9176/2280-6704 ppm	-18 - 6734 / 150 -6494 ppm

There is little difference between the means of the "total population", the "standard population" and the "excluded population". There was greater discrepancy in the ranges of sodium in the "standard" versus "excluded population". This would be expected due to the factors that excluded them from the "standard population" such as receiving fluids, racing or lack of case history.

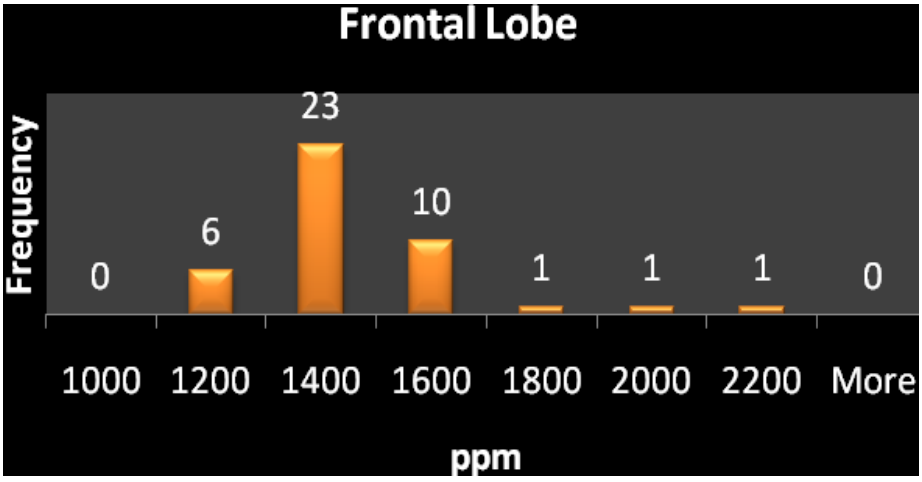
The range and the 95% confidence interval on tables 7 and 8 are not symmetrical. It is suspected that some of the horses may have actually had sodium ion toxicosis. This would skew the range to the right as evidenced by the frequency histogram (charts 1-3) and make the 95% confidence interval not correspond to the range.

With the data collected there is negligible difference in sodium values found on day 1 versus day 2 in mean, range, standard deviation or 95% CI.

Frontal Lobe Data

The mean sodium concentration in the standard population of horses tested in the frontal lobe was found to be 1336 ppm. Sodium concentrations in the frontal lobe ranged from 1039 ppm to 2091 ppm (chart 1). The standard deviation of the frontal lobe sodium data were found to be 194 ppm. Using a 95% confidence interval gave a measured range of sodium in the frontal lobe of 948 ppm to 1724 ppm.

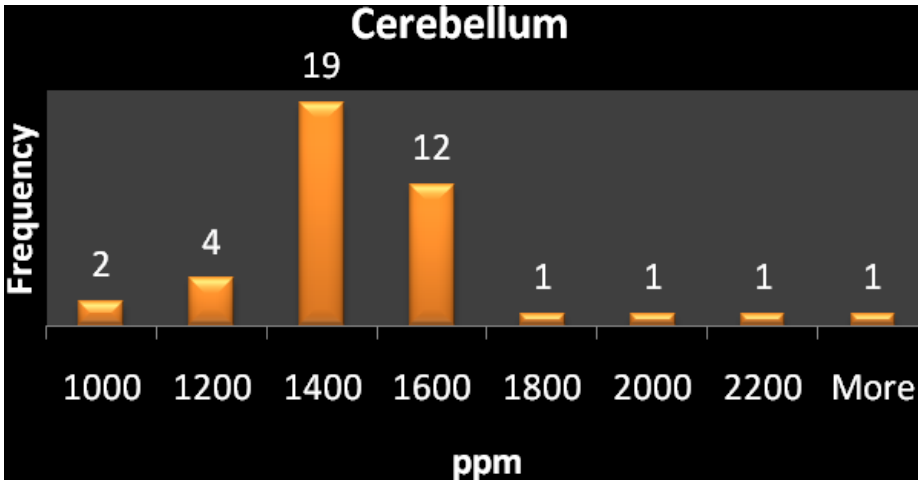
Chart 1: Plotted standard population of frontal lobe sodium values.



Cerebellum Data

The mean sodium concentration in the standard population of horses tested in the cerebellum was found to be 1395 ppm. Sodium concentrations in the cerebellum ranged from 915 ppm to 2552 ppm (chart 2). The standard deviation in the cerebellum was 284 ppm. Again, 2 standard deviations were added and subtracted from the population mean to obtain the 95% confidence interval, making the measured range of sodium in the cerebellum 827 ppm to 1963 ppm.

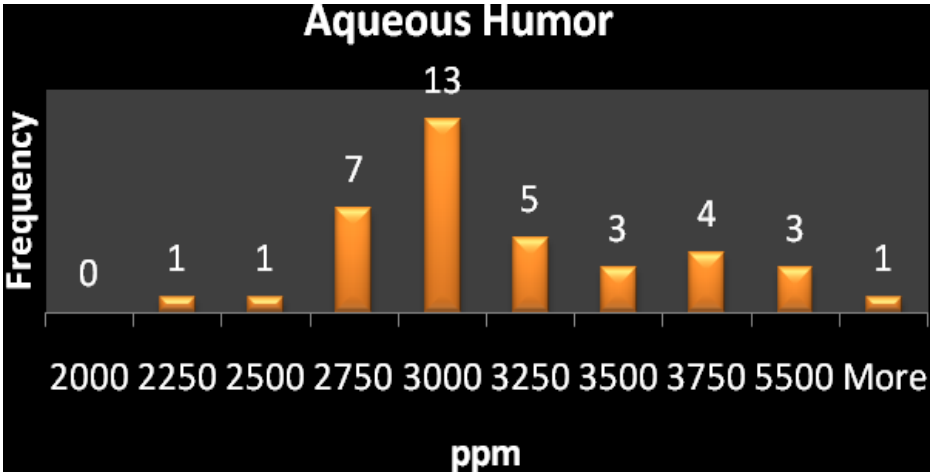
Chart 2: Plotted standard population of cerebellum sodium values.



Aqueous humor Data

The mean sodium concentration in the aqueous humor in the standard population of horses tested was found to be 3147 ppm. The concentrations of sodium in aqueous humor range from 2014 ppm to 4996 ppm (chart 3). The standard deviation in aqueous humor samples is 718 ppm. Adding and subtracting two standard deviations shows normal sodium concentrations in aqueous humor ranged from 1711 ppm to 4583 ppm.

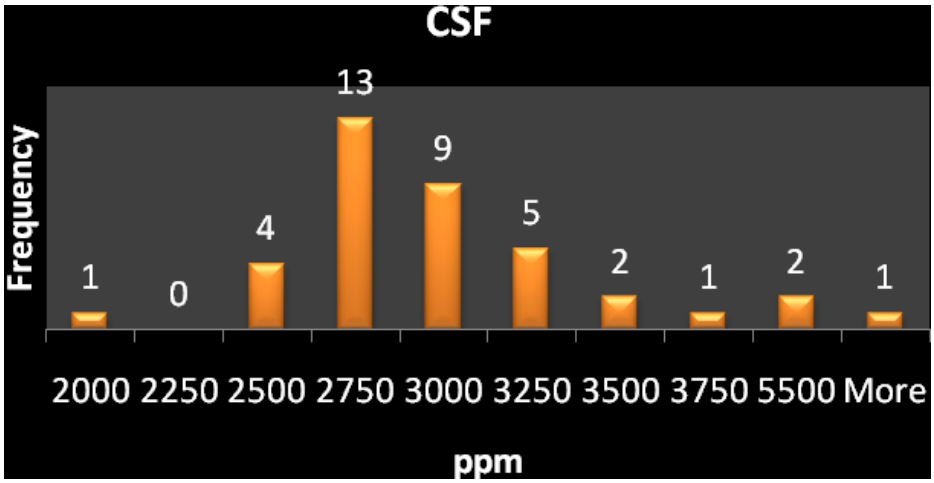
Chart 3: Plotted standard population of aqueous humor sodium values.



Cerebrospinal Fluid Data

The mean sodium concentration in the cerebrospinal fluid in the standard population of horses tested was found to be 3143 ppm. The level of sodium in the cerebrospinal fluid ranged from 1992 ppm to 9176 ppm (chart 4). Standard deviation in CSF samples was 1313 ppm. With this value added and subtracted the normal sodium concentrations in cerebrospinal fluid ranged from 517 ppm to 5769 ppm.

Chart 4: Plotted standard population of cerebrospinal fluid sodium values.



Animals that had sodium values outside of the 95% confidence interval are shown on table 11. The specimen that fell outside the 95% confidence interval is marked on table 11. History, final diagnosis and sodium values for those tissues are also listed on that table. There were not full sets of tissues available for collection on these animals. These tissues would have been useful for comparison. The clinical history and background of these cases were reviewed from submittal forms and hospital records, no fluid therapy or drugs that should affect sodium were noted.

Table 11: Animals outside the proposed reference range including specimen that was an outlier and cause of death

ID #	Frontal Lobe	Cerebellum	Aqueous Humor	CSF	History	Euthanized	Final Diagnosis	Sodium Value
03100279	X	X			Recumbant in stall	Yes	Pulmonary fibrosis	Frontal 2090ppm Cerebellum 2552ppm
03100759	X	X			Dead in pen	No	Suspect allergic rxn	Frontal 1810ppm Cerebellum 2106ppm
04070623	X				Trauma	Yes	Hock Fracture	1764 ppm
03120360		X			Syncope	Yes	Dropped Heartbeat	1875 ppm
03110333		X			Suspect trauma	Yes	Cervical vertebral stenosis	1795 ppm
04011694			X		Sudden death	No	Accute septic shock from ruptured gut	6115 ppm
03091473			X		Donated	Yes	Kyphosis	4989 ppm
03091374			X		Chronic Laminitis	Yes	Chronic laminitis	4690 ppm
05121295			X		Trauma	No	Infected leg and liver	4322 ppm
03082146				X	Epistaxis	Yes	Hemorrhage	9173 ppm
06010379				X	Respiratory Infection	No	Respiratory infection	6690 ppm
04050209				X	Found dead after foaling	No	Hemorrhage	5058 ppm

CHAPTER IV

DISCUSSION

This project was performed to answer numerous requests for brain sodium information on horses submitted to the OADDL. Requests have been made by practitioners and diagnosticians on veterinary message boards and through phone calls, all in reference to cases they had seen or were currently working on. In addition to the requests for this data, a horse was submitted to OADDL for necropsy that presented with a history of sudden death. During necropsy, the pathologist on the case noted abnormally dry gastric contents. The pathologist requested brain sodium analysis, and brain sodium concentrations were subsequently found to be 2010ppm. Since no published normal concentrations were available, this level was compared to those of cattle with comments being made stating this fact. The brain sodium level of 2010ppm was in the toxic range for cattle and was higher than the 95% confidence interval found in the horses in the "standard population" of this study.

In the attempt to establish a reference range, it must be kept in mind that "different methods can be used to establish reference ranges, but all of them begin with the sampling of animals from a healthy population"[31]. Without equine slaughter plants to collect "normal" brains from, the horses necropsied at the OADDL were as close to "normal" as could be collected, although most of these animals could not be considered healthy. At the minimum, it offers a starting place for others who are collecting data or require an estimate of normal brain sodium concentrations in horses.

The primary objective of this study was to determine a typical concentration of brain sodium in the horse and compare the sodium concentrations of frontal lobe and cerebellum. The term "normal" was not used since all of the animals in this study were presented to the OADDL for necropsy subsequent to spontaneous death or euthansia due to health problems. The opportunity was also used to evaluate post mortem CSF and aqueous humor samples and compare these values with previously reported antemortem sodium concentrations.

There were several factors that could attribute to the wide ranges of concentrations that were found in this study. The most likely of these is an inaccurate or incomplete history. It is suspected that injured and sick animals could have been down, without access to water much longer than the submitting client or veterinarian acknowledged, thereby resulting in higher sodium concentrations. This is not unusual, as submitters do not want to appear to have not taken proper care of their horses. The various reasons for death and euthanasia make using the word "normal" impossible. The racehorses that were used in this study died or were euthanized at the racetrack. Besides being stressed, these horses may have been given various drugs, such as furosemide, that were not acknowledged in the case history.

Another problem with this study was the inability to collect large volumes of normal samples from slaughter houses which can be done in the cases of cattle and swine. Without equine slaughter facilities, the availability of a high volume of "normal" samples that came through the diagnostic lab was limited. There were outliers that made some of the results questionable. Such outliers represent the true range of values that are expected to occur in a necropsy setting. Were samples able to be collected from a slaughter

facility, where horses were actually walking; there would be a much smaller range in measured sodium concentrations with fewer outliers.

There is always a possibility of human error in any research. Although the analysis was performed by a trained technologist with 8 years of experience, and all possible measures were taken to remove sources of error, some of the variability in samples cannot be explained, in particular CSF.

CSF, aqueous humor, cerebellum and frontal lobe results will be discussed individually. They will each include a discussion of the results for the total population, results for the "standard population", a brief comparison to see if the day of necropsy affects the concentration of sodium and a comparison of sodium concentrations known in other species, specifically cattle and swine.

The sodium concentrations in the CSF from the "total population", in which several variables were compared, had no significant differences with the one exception of the effect of breed of horse. The data shown in table 6 demonstrates that there was a significant difference between thoroughbreds and "other breeds" of horses. Where the sodium concentration found in the CSF were higher in the thoroughbreds. The thoroughbred population was predominately racehorses as 33 of the 37 thoroughbreds were racehorses, compared to 8 of the 40 horses in the "other breeds" category that were racehorses. However, there was no significant difference in the CSF between quarter horses and thoroughbreds, or quarter horses and "other breeds" of horses, denoted by the A and B superscript. Because many of the thoroughbred horses were racehorses, this might explain why the thoroughbred CSF sodium concentrations were higher than "other breeds".

The standard population consisted of 39 CSF samples with a range of 1992-9176 ppm (table 7). Three of the CSF samples fell outside of the 95% confidence interval (table 11). One of the reasons for the wide range in this study was due in part to 3 horses with abnormally high sodium concentrations in the CSF. After reviewing these 3 cases, 2 with a history of hemorrhage and 1 with a respiratory tract infection, further study might suggest a reason as to why these CSF sodium concentrations were high. There were no brain samples available from these 3 horses to support or contradict these high CSF sodium findings. The two highest CSF samples (9173 ppm and 6690 ppm) would be considered outliers by the rule-of-thumb test mentioned and reference in the statistics section of the materials and methods chapter. By removing these 2 values from the standard population, it gives a new range of 1992 ppm to 5057 ppm. Even with the removal of 2 highest concentrations, this range is still quite wide and should be studied further before it is used for diagnostic purposes.

Possible reasons as to why some of these specimens were outliers could be the undocumented use of drugs such as phenylbutazone which can decrease blood flow to the kidneys thereby causing water retention and sodium retention. Furosemide is another drug that can lead to dehydration and cause electrolyte imbalance20. There could also be undocumented use of fluids that could affect sodium concentrations. The most likely suggestion is that the animals were without water longer than suspected.

Since CSF values have been tested in the live animal, the CSF values in this study were used to compare the antemortem versus postmortem concentrations of sodium. The range found in this study was 1992-9697 ppm in the standard population. The normal range of sodium in the CSF in the live horse is 3219ppm- 3448ppm [22, 28]. The 2 studies

that report these values were done on live horses. It is not surprising that dead horses, with a wide variety of history, have a much wider range. The reference range for normal, live cattle and swine is 2988-3563 ppm (table 1).

On table 10 there is a comparison of animals that were necropsied on day 1 versus day 2. Twenty five animals in the standard population that had CSF harvested, were necropsied on day 1 and 17 on day two. There was no difference in mean value, range or 95% confidence interval for the CSF suggesting time of death under 48 hours did not have an impact on sodium levels.

In hindsight, CSF samples should not have been taken without a brain sample to compare them to. At this point all that can be done is to refer back to the reasons for possible error with incomplete history being high on the list.

The aqueous humor sodium concentrations from the total population of horses, including all of the variables had no significant differences with the two exceptions of horses that had been on fluids and breed of horses. The same explanation for the significant difference found between thoroughbreds and "other breeds" could be used to explain the higher sodium concentration in the aqueous humor. It is not surprising that the administration of fluids to an animal affects the concentration of sodium. The aqueous humor had a P value calculated at 0.0515. This is greater than the 0.05 P value that was used in determining a significant difference. However, since this is only slightly greater than 0.05 it would warrant further study to determine if there actually was a significant difference.

The 95% confidence interval of sodium concentration in the standard population was 1711 – 4583 ppm in the aqueous humor. The range in this study, compared to the

range found in the McLaughlin study (2689 – 3218 ppm) was wider; however the samples in the McLaughlin study were collected from a slaughter facility [23]. The mean aqueous humor sodium concentration determined from a live horse is 2698 ppm [24].

In comparing aqueous humor sodium concentrations from the standard population to other species, there were no aqueous humor sodium values reported in swine. The range of aqueous humor sodium concentrations for cattle is 2966-3586 ppm[4]. This was data compiled from 8 animals that, like this study, were not normal, but not suspected of water deprivation[4]. The range for aqueous humor sodium concentration in cattle falls within the 95% confidence interval for the standard population of horses 1711- 4583ppm. This 95% confidence interval is quite wide and suggests that the sample can be quite variable and is not be the best sample to test for post mortem sodium concentrations. The 4 highest aqueous humor values, although they do not qualify as outliers by the previously mention test, do not have brain tissue to compare. Brain tissue in these animals would have been useful for comparison to see if the brain also contained elevated levels of sodium.

The McLaughlin study shows that sodium concentrations began to decline significantly between 24-48 hours and they suggest that the sample might be considered unreliable[24]. Other sources suggest that the aqueous humor remains stable postmortem up to 24 hours[4]. This may help explain why the range in this study was so wide (table 10). Horses in this study were not from a slaughter facility and while the aqueous was collected within 48 hours of arrival to OADDL, the time of death of the animal is subjective at best. This information would suggest that sodium in aqueous humor is most reliable when tested within 24 hours regardless of storage temperature. This study

showed the range was wider on samples collected at 48 hours than samples collected within 24 hours, showing the unreliable nature of the sample.

The cerebellum sodium concentration from the "total population" of horses found no significant difference in sodium concentrations regardless of variable. The cerebellum sodium concentration range in the "total population" was 636 – 4933 ppm. The 95% confidence interval for the "total population" was 391 – 2399. This range is much wider than the range found in the "standard population". The "standard population" sodium concentration range was 915 – 2552 ppm, with a 95% confidence interval of 827 – 1963 ppm. There is no way to compare cerebellum sodium values with other species because no information could be found. Other studies do not specify what part of the brain was tested. Cerebellum data will be compared to frontal lobe values. There appears to be no difference in any values when comparing day 1 versus day 2 cerebellum sodium concentrations (table 10).

The frontal lobe sodium concentration from the "total population" of horses found no significant difference in sodium concentrations regardless of variable. .The mean level found in the frontal lobe of horses was 1326ppm for the "total population". The range for the "total population 890 - 2453 ppm with a 95% confidence interval of 714 – 1958 ppm sodium.

The sodium concentration in the frontal lobe of the "standard population" ranged from 1030 – 2090 ppm sodium. The 95% confidence interval was 948 – 1724 ppm sodium. The range and 95% confidence intervals are similar between the frontal lobe and the cerebellum. This suggests that it is the site of harvest for brain tissue is inconsequential.

There were 3 frontal lobe values that fell outside the 95% confidence interval. Using the rule-of-thumb for dealing with the dilemma of outliers in the frontal lobe, the distance between the highest value (2090ppm) and the 2nd highest (1810ppm) gives a difference of 280ppm[31]. This distance did not exceed one-third of the range of all values (1051/3=350). These higher values cannot be eliminated by this rule. Using the same rule of thumb on the four suspected outliers in the cerebellum, they show they should not be excluded as outliers. It is possible that the suspected outliers on the frontal lobe and cerebellum may have been down and without water longer than reported.

The most informative reference quoted for sodium concentrations in cattle tested brain sodium concentrations in 100 animals [15]. The majority of these were collected from a slaughter facility, with some being collected from a diagnostic lab. The normal cattle brain sodium range found in that study is 701-1349 ppm with a mean value of 1025[15]. The normal range of brain sodium concentration in swine is 1850 – 2030 ppm[3]. Another reference states 1800 ppm as a normal level for swine[1]. The mean values of the cerebellum and frontal lobe in the horse (1395 and 1336 respectively) are lower than the normal reported values for swine but higher than the reported normal for cattle. When comparing the 95% confidence interval from the frontal lobe (948-1724ppm) from the "standard population" of horses to the ranges listed for cattle and swine (table 1), the range is higher than cattle (701-1349 ppm) but broader and lower than those in swine (1850-2030 ppm).

There appears to be no difference in sodium concentration in the frontal lobe when comparing day 1 versus day 2 necropsy in the standard population. There is no

available data on brain sodium values in cattle and swine when comparing necropsy on day 1 versus day 2.

In summary, the range of sodium concentrations and 95% confidence intervals in the aqueous humor and CSF found in this study were too wide to be useful in a diagnostic laboratory. The reasons for this variability have already been mentioned. At this time, there are no diagnostic laboratories that report a "toxic" level of sodium in aqueous humor and it is not routinely tested in toxicology laboratories reiterating that it is unreliable after 24 hours.

The brain ranges were wider than those reported for cattle and swine. This is not surprising since millions of normal cattle and swine are slaughtered every day and samples have been tested over the years and only thirty nine were examined in this study. Even though the mean of the cerebellum was similar to the frontal lobe, the range and 95% confidence interval was wider, making it less ideal than the frontal lobe for diagnostic purposes.

It was determined that the brain frontal lobe was the most reliable sample to test for sodium concentrations. The 95% confidence interval from the standard population was realistic compared to normal ranges for other species.

The original horse that presented at OADDL had a brain sodium level of 2010 ppm and was considered "toxic" by cattle standards. The information collected during this study established a frontal lobe range of 948 – 1724 ppm sodium. Although the horse normal range was found to be slightly higher than cattle, this horse would still be considered highly suspect of a water deprived animal. To obtain a definitive diagnosis more studies would need to be performed to determine what a toxic level of sodium

would be in the horse. As this type of study would be considered inhumane, the only other option would be to collect and analyze specimens from known water deprived horses that are presented to the diagnostic lab.

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VITA

Kelly Anne McCracken

Candidate for the Degree of

Master of Science

Thesis: A study of postmortem sodium concentrations in the Brain, Aqueous humor, and Cerebrospinal fluid of Horses received at Oklahoma Animal Disease diagnostic Laboratory

Major Field: Veterinary Biomedical Science

Biographical:

Personal Data: Born in Tulsa, Oklahoma, on October 6, 1977, the daughter of James R. and Nanette McCracken.

Education: Graduated from Charles Page High School, Sand Springs, Oklahoma in May 1996; received Bachelors of Science in Animal Science from Oklahoma State University in December 2001; enrolled in the Master of Science in Veterinary Biomedical Science program at Oklahoma State University in August 2003; completed requirements for Master of Science degree in December, 2009.

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Name: Kelly Anne McCra Date of Degree: December, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: A study of postmortem sodium concentrations in the Brain, Aqueous humor, and Cerebrospinal fluid of Horses received at Oklahoma Animal Disease diagnostic Laboratory

Pages in Study: 53 Candidate for the Degree of Master of Science

Major Field: Veterinary Biomedical Science

Scope and Method of Study:
Water deprivation/sodium ion toxicosis is well documented in certain species. A normal sodium range as well as a toxic range has been previously established for cattle, poultry and swine. Diagnostic laboratories often receive requests for brain sodium levels to use in conjunction with histological findings for diagnosis of water deprivation/sodium ion toxicosis. No reference values for equine brain sodium concentrations have been found in the literature.

Aqueous humor, cerebrospinal fluid, cerebellum, and frontal lobe specimens were harvested from horses with a history that did not suggest a neurologic problem and that were submitted to Oklahoma Animal Disease Diagnostic Laboratory for necropsy. Testing was done at Oklahoma Animal Disease Diagnostic Laboratory in the toxicology laboratory using atomic absorption spectrometry to determine sodium levels in the frontal lobe, cerebrospinal fluid, aqueous humor, and cerebellum of the horse.

Findings and Conclusions:
Samples were tested and statistically show that the probable range for sodium in the horse is 984 ppm to 1724 ppm in the frontal lobe, 827 ppm to 1963 ppm in the cerebellum, 75-199 mEq/L (1711 ppm to 4583 ppm) in the aqueous humor, and 22-250 mEq/L (1992 ppm to 9697 ppm) in the CSF. The brain values will be very useful to veterinarians and diagnosticians across the country. With the increased public awareness of cruelty/neglect cases, these values will allow for more accurate diagnosis of water deprivation/sodium ion toxicosis in horses.

ADVISER'S APPROVAL: Sandra Morgan