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Seroprevalence and Titer Concentration Testing for Leptospirosis in Equine

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SEROPREVALENCE AND TITER CONCENTRATION TESTING FOR LEPTOSPIROSIS IN EQUINE

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Abstract

The most common best management practice used to combat diseases in horses is vaccination, which can decrease the incidence or severity of illness. However, the duration of immunity will vary for each vaccination and must be considered for revaccination purposes. Titer concentration, which evaluates antibodies in the blood, may be a helpful way of determining how long an animal has immunity to a disease. Leptospirosis, a zoonotic, bacterial disease, can result in uveitis, potentially leading to blindness, and abortion in mares. One serovar, Leptospirosis pomona (L. pomona), is associated with most cases of clinical disease in horses in North America. There is one approved vaccine, specific for L. pomona, currently available for this disease. The objective of this study was to evaluate the immune response in horses to the L. pomona vaccine.

Forty previously unvaccinated horses from the Murray State University Equine Center were used in this study. None had previously documented issues with uveitis or abortion. Blood was drawn and serum removed for evaluation of leptospirosis titers. Horses were then divided into TRT (vaccinated, n=20) or CON (not vaccinated, n=20), and TRT horses were vaccinated for leptospirosis. Blood samples were collected from 37 (TRT n=20; CON n=17) horses 14 d post-vaccination. Horses in the TRT group received booster vaccines 3 weeks after the first vaccination. Blood samples were again collected from 37 horses 14 d post booster. At each collection, 20 ml of blood were collected by jugular venipuncture into 2 red top vacutainers® tubes. Samples were centrifuged and

serum removed within 24 hours. Serum was delivered to the Breathitt Veterinary Center and either analyzed within 48 hours or frozen until analysis could be completed. Descriptive statistics were used to evaluate the results.

Preliminary results showed a higher seroprevalence of L. pomona in this study (8%, n=3 of 37) when compared to previously published research of unvaccinated horses. Kitson-Piggot and Prescott (1987) found that only 4% of horses in Ontario had a positive titer for L. pomona, and Fagre et al. (2020) found only a 1% incidence for the same serovar in horses in Colorado. Given that the location for horses in the previous studies were spread out over a larger geographical distance than the horses in this study, it is possible that the University horses were concentrated and exposed to a greater degree than horses in the other studies. After the initial vaccination was administered, all horses in the TRT group showed an increase in antibody concentration which resulted in a positive titer for each horse. Titers increased at varying rates, ranging from 1:200 to 1:25600. After receiving the booster vaccination, five TRT horses showed no change in titer, six horses had another increase, and nine horses showed a decrease in titer concentration. No horses showed any signs of illness related to leptospirosis during the study. Further analysis of the results may lead to greater understanding of the equine immune response to this vaccine.

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Literature Review

Introduction

Vaccines are given to most, or all, domesticated animals to aid in protection or prevention of diseases. Currently, most vaccines are given on an annual basis, but studies have led us to believe that this may not be necessary as the immunity, or the titers, can last longer than a year with some vaccines in some animals (Moore & Glickman, 2004). This new development in vaccination guidelines has led to more studies on vaccination immunity length in different animals. This literature review focuses on equine vaccinations and their uses, and the vaccine information and guidelines for leptospirosis in equines.

Diseases

According to the Merriam-Webster dictionary, a disease is anything that impairs the normal function of a living thing[1]. The Mayo Clinic states that infectious diseases are disorders caused by organisms like bacteria, viruses, fungi, and parasites[2]. Common diseases in horses include equine influenza, tetanus, and equine encephalomyelitis. A variety of methods can be used to protect animals from diseases. This can include

[1] Merriam-Webster. (n.d). Disease. In *Merriam-Webster.com dictionary*. Retrieved September 10, 2022, from https://www.merriam-webster.com/dictionary/disease.
[2] Mayo Clinic. (2022, February 18). *Infectious diseases*. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/infectious-diseases/symptoms-causes/syc-20351173

biosecurity measures, such as isolation of sick animals or proper manure management, as well as vaccinations.

What are Vaccines

A vaccination is a product that has been developed to give an animal immunity against a disease or virus. They help to prevent or reduce the effects of certain diseases as well as protect against transmission of zoonotic diseases. When an animal is vaccinated, antibodies are formed to combat the pathogens simulated by the vaccine. The animal then has what is called active immunity. Active immunity is obtained through vaccinations and protects the animal against the disease or virus. As the animal ages and comes in contact with these pathogens in a live situation, their body will already have the antibodies to protect them against contracting it, or in other cases, greatly reducing the symptoms (Scott, n.d.).

Types of Vaccines

Vaccines are currently available as modified live vaccines or inactive vaccines. Modified live vaccinations contain live, attenuated organisms, or a weakened form of the virus. They create an immune response in the animal that replicates the response if the animal were to actually contract the disease. This immune response allows for the rapid production of antibodies and gives the animal protection against the virus in the future. Inactive vaccines contain viruses that have been exposed to inactivating agents like formaldehyde, β-propiolactone, ethylene-imine, or thimerosal (Minke et al., 2004). Immunogens are then extracted from the inactive virus and combined with adjuvants to

create the vaccine. The immunogens and adjuvants stimulate the antibody production for the virus in the animal's body. The downsides to inactive vaccines are that their antibody production is much slower compared to the production rate when modified live vaccines are used. Additionally, they often require boosters and do not create a strong immunity to the virus (Minke et al., 2004).

Vaccinations can also have adverse effects on an animal. Most vaccines will produce some kind of reaction either around the injection site or in mild clinical signs, but this is to be expected after stimulating the immune system so strongly with the vaccination. More severe adverse reactions can be caused from improper handling or administering of the vaccine, contamination, allergens, and other causes. Some vaccines have also failed to protect the animal from the disease. Vaccine failure can be caused by things like insufficient time to develop immunity, alterations to the vaccine, host factors preventing immunity, excessive exposure to pathogens, and other causes (Roth, 1999).

Most vaccines continue to be administered on an annual basis, while others are given more or less frequently. Factors affecting vaccine frequency can include risk level in the area, duration of immunity for that vaccine, the animal’s response to the vaccine, etc. Vaccinations are classified as either core vaccines that are given every year in every location, or risk-based vaccines that are given on an as needed basis. Equine core vaccines include tetanus, Eastern and Western encephalomyelitis, rabies, and West Nile virus (Desanti-Consoli et al., 2022). These diseases are core because some are incurable and some are zoonotic, meaning they can infect other species including humans. Risk based vaccines are given based on location and risk level (Desanti-Consoli et al., 2022).

Some locations have a higher prevalence of diseases compared to other locations. Additionally, some animals may be more susceptible, or at a greater risk to some diseases. In these situations, risk-based vaccines relevant to each animal's needs and location would be administered.

Duration of Immunity

Duration of immunity can be affected by a number of variables. Whether the vaccine is live or inactive, the disease that the vaccine is for, and the age of the animal are a few factors that can change the duration of immunity. For example, in dogs, immunity from vaccinations has been shown to last up to three years for certain pathogens (Harvey et al., 2016). Additionally, geriatric animals may have a decreased immune response to vaccines (Harvey et al., 2016). Due to the wide number of variables that can affect the duration of immunity, determining an accurate vaccination timeline for every horse can be difficult. By not revaccinating every year, it can help to reduce adverse symptoms created by vaccinations but changing vaccination intervals could leave some animals unprotected against these diseases. Blood tests to measure the titer levels can be a way to help determine how often a horse, or any animal, may need to be revaccinated.

Titers

Titers are the concentration of antibodies found in the blood. Testing can be done to determine titer levels, and titers can then aid in determining the level of immunity an animal has against a disease. Studies have been done using titer testing to try to redefine the vaccination guidelines in animals (Burr, 2006). The issue with this is that

uncontrolled variables, such as environment or at home care, as well as different techniques between veterinary practices make interpreting titers on a large scale difficult. Therefore, to change the vaccine intervals on a universal level could leave some animals unprotected if vaccinations were done less frequently (Burr, 2006).

In horses, titer evaluation can be useful for the efficacy of some vaccines such as rabies, but with others like Eastern/Western encephalomyelitis and West Nile virus, the titer levels needed for protection are not clear. Additionally, for diseases like equine influenza and equine herpesvirus which transmit through the respiratory tract, local immune response is more important than blood antibody levels[3]. For example, the local immune response to the influenza vaccine would be the production of virus-specific, antibody-secreting cells in the respiratory system (Brokstad et al., 1995). These cells are more important in the defense against influenza than the titer concentration in the blood. In 2002, the AVMA reported that titer testing in place of vaccinations is generally unreliable (Moore & Glickman, 2004). This is due to the lack of knowledge on what levels are actually needed to maintain protection for the animal.

Leptospirosis

Leptospirosis is a bacterial disease that affects both animals and humans. The bacterium, Leptospira, has over three hundred serovars with Leptospirosis pomona (L. pomona), being the most common in horses (Fagre et al., 2020). Other serovars

[3] The case for (and against) pre-vaccination serology. (2021, May/June). Insight Equine Edition, 12-14.

commonly tested for in horses are Leptospirosis grippotyphosa (L. grippotyphosa), Leptospirosis icterohaemorrhagiae (L. ictero), Leptospirosis canicola (L. canicola), Leptospirosis hardjo (L. hardjo), and Leptospirosis bratislava (L. bratislava). In equine, the disease is often transmitted through bodily fluids, contaminated water sources or land, through the mucus membranes, and through wet or damaged skin. The disease can affect kidneys, eyes, and pregnant mares causing acute renal failure, abortion, recurrent uveitis, and many other issues. One vaccination has been approved for use in horses called the Lepto EQ Innovator®. This vaccination is an inactive or killed vaccine that targets the L. pomona serovar only. For this vaccine to be effective, it must be administered to horses not carrying any active or dormant Leptospira bacteria (Divers et al., 2019).

The titer antibody test for Leptospirosis is called the microscopic agglutination test (MAT). The titer reading for this vaccine can vary widely, and the duration of immunity is unknown for the vaccination. The Lepto EQ Innovator vaccine is currently given on an annual basis, with the belief that it provides immunity for at least a year after administration (Divers et al., 2019). In dogs, an acute infection can be present, and the MAT test will still read negative. Additionally, no difference is shown between titers from infected dogs and vaccine induced titers making this test challenging to interpret. It is said that using MAT titers to determine the vaccines duration of immunity is known to be inaccurate, with several studies being done that show no relation between the vaccine titers and actual protection from the disease, although this is based on studies conducted on dogs (Martin et al., 2014). There may prove to be a difference when a similar study is conducted on horses.

Summary

The current belief that leptospirosis titers have little to no effect on the horse's immunity to the disease is still not a proven fact. Titer testing is currently the most accessible and common way to test immunity for Leptospirosis. If a specific titer range can be found that guarantees immunity the vaccination guidelines could be more accurately provided. This study will aim to either support or disprove this belief that titers have little effect on the horse's immunity to leptospirosis. This will be done through lab testing on a number of horses.

Materials and Methods

This project was approved by the Murray State University Institutional Animal Care and Use Committee with the protocol number 2023-020.

Project 1 Baseline Comparison

Thirty-seven horses, both geldings (n=28) and mares (n=9), between 4 and 26 years old were selected from the Murray State University equine herd. The horses were used regularly in the program as mounts for classes and team practices. They were housed in the university barn or in the pastures at the Murray State Equine Center. The horses received routine management including daily general care, regular vet care as needed, and annual vaccinations continued during the project.

On day zero of the study, two 10 mL Vacutainers® (BD Vacutainers, Franklin Lakes, New Jersey, USA) of blood from each horse via the jugular vein. These samples were placed on ice until being transported to Breathitt Veterinary Center. Samples were then placed in a Thermo Scientific Multifuge X3 machine where they were spun at 3300 rpms for 5 minutes. This allowed for the serum to be removed from each sample. The serum was analyzed using the microscopic agglutination test to assess titer concentration for five leptospirosis serovars: L. pomona, L. ictero, L. grippotyphosa, L. canicola, and L. hardjo. For the purpose of this study, horses were considered positive if titer concentrations were at or above 1:200.

Project 2 Vaccination and Booster

Horses from Project 1 were split into two groups, TRT (vaccinated, n=20) and CON (not vaccinated, n=17). Horses in the control group remained unvaccinated for the study and served as a baseline to compare to those vaccinated. The treatment group of horses were vaccinated by a licensed veterinarian using the Lepto EQ Innovator® (Zoetis LLC, Parsippany, New Jersey, USA) on day seven. Each horse received the recommended, intramuscular 1 mL dose of the vaccine on the left side of the neck. All TRT horses were geldings with no previous history of leptospirosis or related diseases. Horses were monitored for any adverse reactions following the vaccination.

On day 21, blood was drawn from all 37 horses with the intent to track changes in TRT horses after vaccination. Samples were collected and processed as described in Project 1.

According to manufacturer's guidelines, the Lepto EQ Innovator® requires a 1 mL booster dose three to four weeks following the initial vaccine. On day 28, TRT horses received the recommended booster vaccination. The vaccine was given on the left side intramuscularly and was performed by the same veterinarian as in Project 1. All vaccinated horses were again monitored for any adverse reactions following this vaccination.

On day 42, blood was drawn again on all 37 horses to evaluate any changes after the booster vaccination. Samples were gathered and processed as described in Project 1.

Statistics

Descriptive statistics were used to evaluate the data. Figures were generated using Microsoft Excel (Version 16.66.1). Reported titer concentration values were graphed, as were changes over time (no change, increase, or decrease).

Results and Discussion

No horses exhibited any symptoms related to adverse vaccine reaction or to leptospirosis infection during this study. There is limited published research on leptospirosis titers in horses. Cut offs used in different equine studies have ranged from 1:80 to 1:200. The laboratory used to evaluate this data did not evaluate ratios below 1:100. Based on previous publications, this study used a cut off 1:200 to indicate a positive titer.

Project 1 Baseline Comparison

Overall, results from this study showed that a majority of the Equine Center's herd had been exposed to leptospirosis at some point. Using the cut off 1:200 to indicate a positive titer, 86% of the University's horses were considered positive for at least one serovar (Figure 1). The highest incidence was with L. ictero (83.7% positivity rate).

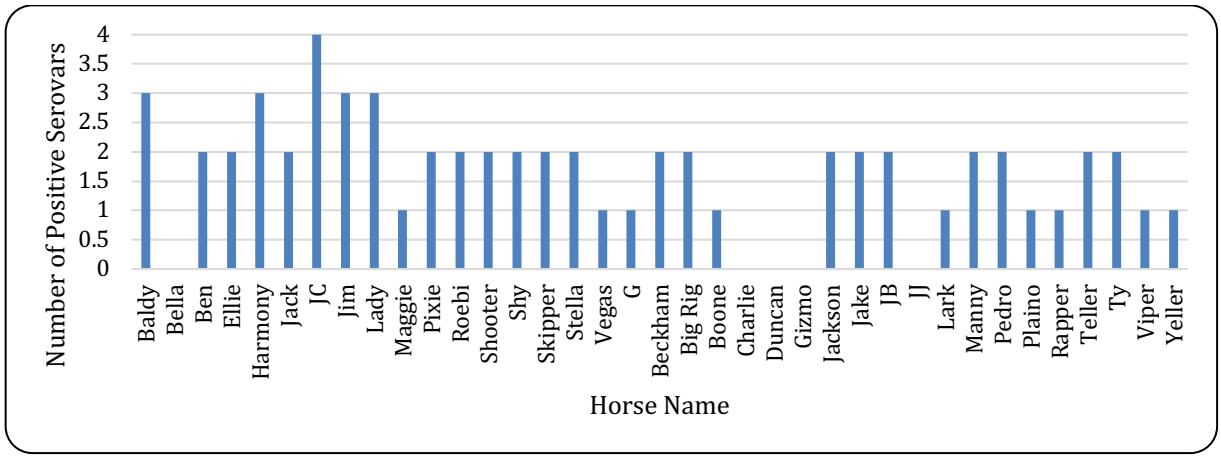

Figure 1. Number of positive titers in unvaccinated horses for one or more Leptospirosis serovars at day 0 (baseline testing).

Previous work has shown some differences as well as similarities to the current study. Kitson-Piggot & Prescott (1987) evaluated 557 horses in Ontario for previous exposure to leptospirosis and showed a 33.9% positivity for a titer of 1:80 or above. The most prominent serovar was L. bratislava, with 27.5% of the horses showing a positive titer of 1:80 (Kitson-Piggot & Prescott, 1987). A similar study by Farge et al. (2020) evaluated 124 horses from various regions in Colorado. Of the 124 tested, 82% showed a titer of 1:100 or higher, and 57% showed a titer of 1:200 or higher, for one or more serovars. The most prominent serovar was again L. bratislava, with 67% having a titer of 1:100 or more, and 37% with a titer of 1:200 or more (Farge et al. 2020). Also, the current study did not have the opportunity to test for L. bratislava, therefore, no conclusions could be determined. However, Farge et al. (2020) showed that horses with a high titer for L. ictero also had high titer for L. pomona, which agreed with this study (Figure 1). Between these three studies, there were distinctly different results in the titers of previously unvaccinated horses. It is possible that the leptospirosis bacteria are becoming more prevalent in the environment, and that fewer horses had been exposed in 1987 as compared to 2020, or even 2022. Another consideration could be geographical location. The locations of the previous studies have completely different climates compared to western Kentucky. Ontario and Colorado both experience hard freezes that may impact the lifespan and concentration of Leptospirosis bacteria in the area, therefore potentially reducing the seroprevalence. Additionally, horses in both of the previously published studies were spread out over a larger geographical area than horses in this study. It is also possible that the University horses were concentrated and exposed to the leptospirosis bacteria to a greater degree than horses in the other studies.

Project 2 Vaccination and Booster

Horses in this study received a vaccine that specifically targeted L. pomona. After the initial vaccination was administered, 100% of the horses in the TRT group showed an increase in antibody concentration for L. pomona. This resulted in a positive titer for each horse, which was expected. Leptospirosis pomona titers increased at varying rates, ranging from 1:200 to 1:25600 (Figure 2).

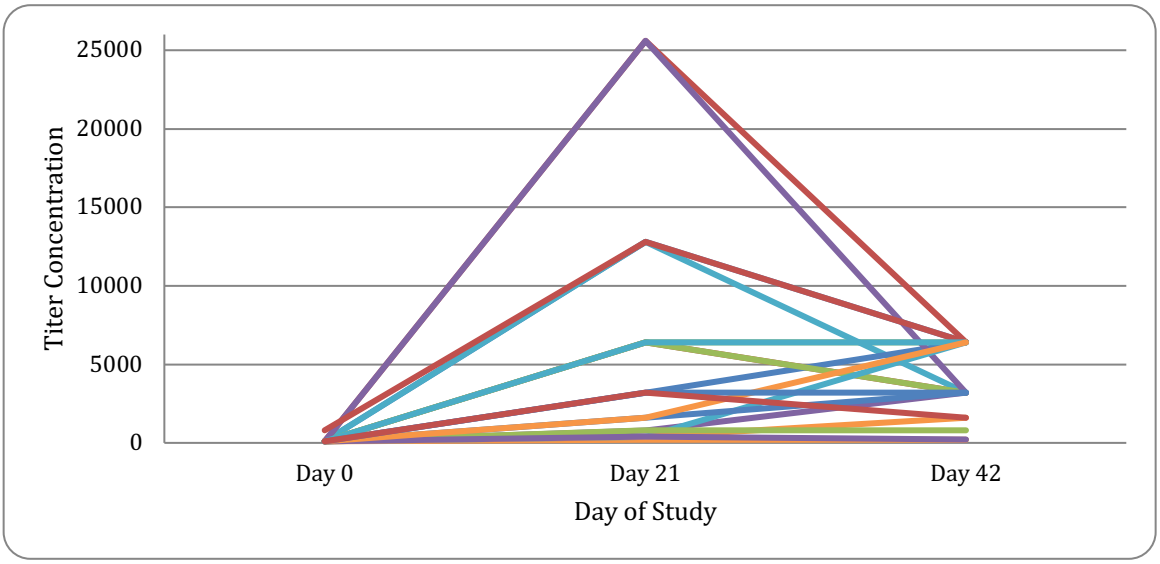

Figure 2. Changes in Leptospirosis pomona serovar titer concentration in horses 14 days after first vaccination (day 21 of the study) and then 14 days after booster vaccination (day 42 of the study). Horses were vaccinated on day 7.

Following the booster, 45% of the TRT horses had a decrease in their titer concentration, 30% had an increase, and 25% had no change (Figure 3). A previous study on the titer response to West Nile virus also showed a decrease in titers after the initial vaccine but before the booster (Khatibzadeh et al., 2015). This study vaccinated horses on day 0, tested titers on day 14 and day 46, boostered on day 46, and then tested titers again on day 74. Results showed that the titer levels had reached a peak at day 14 and then began to decrease before the booster was administered on day 46. The titer then peaked again on day 74, and they were higher than the peak before the booster (Khatibzadeh et

al., 2015). This current study did not test again until after the booster vaccination, so it is unknown if the titers began decreasing before the booster or after. It is also possible that the timeframe for titer testing this study did not allow enough time for the titers to fully rise again after the booster. Further testing in the future may allow for a better understanding of the physiology behind these unexpected changes.

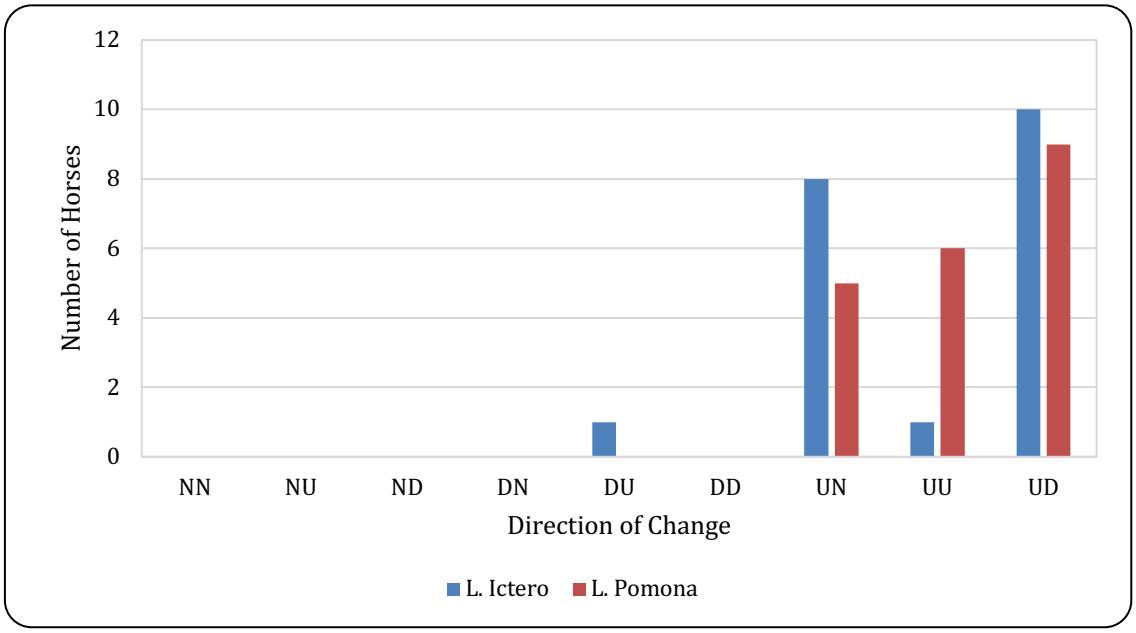

Figure 3. Changes in Leptospirosis pomona and Leptospirosis ictero titer concentrations after vaccination (first letter) and after booster (second letter) in treatment group horses. Letters indicated increase (U), decrease (D), or no change (N).

While the vaccine was targeting L. pomona, it is possible it had some effect on other serovars. Figure 3 displays the changes for L. pomona and L. ictero after vaccination and booster (N= no change, D= down/decrease, U= up/increase). With the vaccination, 95% of the TRT horses also had an increase in the L. ictero serovar (Figure 3), with 100% carrying a positive titer for this serovar. It is interesting to note that L. ictero titers increased numerically when horses were vaccinated with the L. pomona vaccine (Figure 3).

As with L. pomona, many of the horses had a decrease in titer concentration for L. ictero following the booster vaccination. Similar studies have also shown a correlation between L. ictero and L. pomona (Farge et al., 2020). Breathitt Veterinary Center staff confirmed that these two serovars are closely related or work with each other, and therefore fluctuate similarly (K. Doctorman, personal communication[4]).

The L. hardjo titers for the TRT horses remained unchanged in all horses except for one. After receiving the Lepto EQ Innovator®, one gelding in the TRT group produced a positive titer for L. hardjo. This horse's titer then dropped below the 1:200 cut off and was no longer positive after the booster vaccination when tested on day 42 (Figure 4). It is likely that this is unrelated to the vaccination as there is no correlation between the L. hardjo and L. pomona serovar and the vaccine does not target L. hardjo.

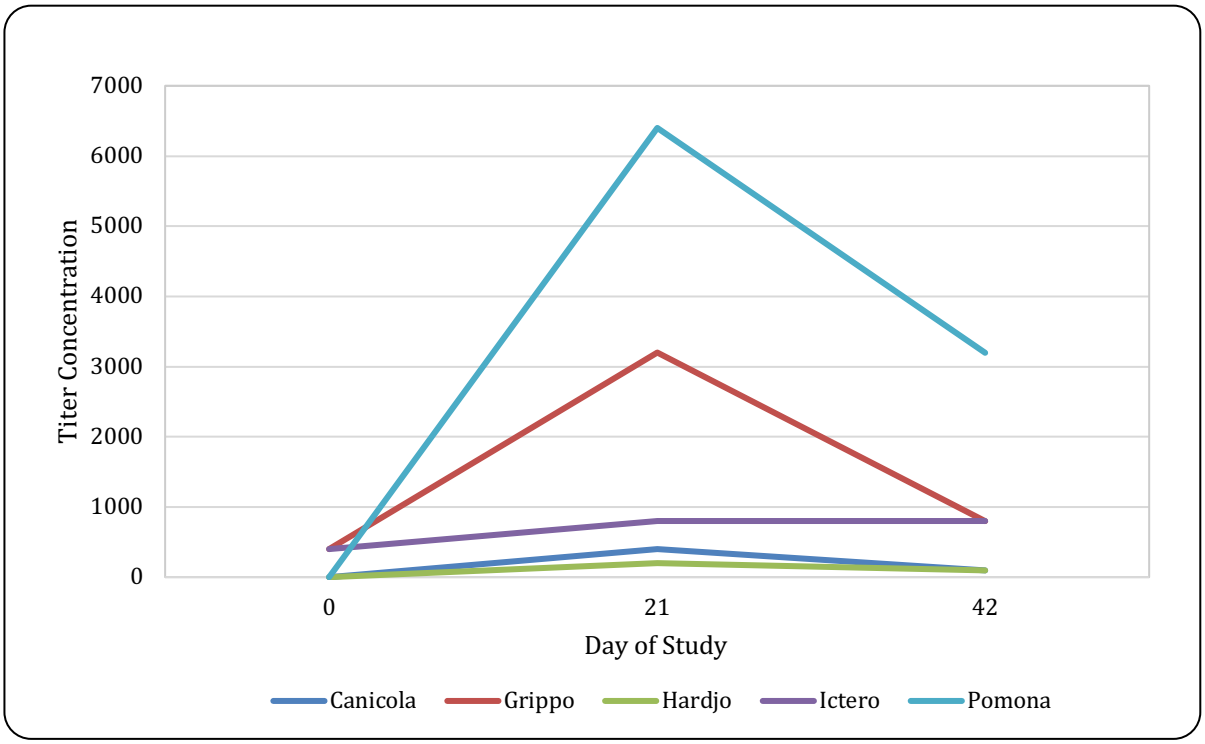

Figure 4.One horse's titer concentration changes for each Leptospirosis serovar at day 0, day 21 (14 days after vaccine), and day 42 (14 days after booster).

[4] K. Doctorman. Murray State Breathitt Veterinary Center, Hopkinsville, KY. 2022.

For the serovar L. grippotyphosa, TRT horses had a lot of variability in titer concentration response with no clear pattern in the changes. A majority of the TRT group saw some increase in their L. grippotyphosa titer levels after the vaccine. There was a strangely large jump from 1:400 up to 1:3200 in one horse's L. grippotyphosa titer that then drastically decreased after the booster (Figure 4). This horse showed similar results for its L. pomona titer. This horse was the same one noted for different L. hardjo changes.

For L. canicola, the TRT group showed a lot of variation post initial vaccine and booster titers. A third of the horses had no change at all, even after the vaccine and booster were administered. Another third had an increase in their titer levels after the vaccine, but the titer dropped after the booster. The remaining horses had some kind of change, either up or down after the vaccine and booster. One horse, different from the one referenced for different changes in L. hardjo and L. grippotyphosa, had the highest titer at the baseline for L. canicola, but concentrations dropped after the initial vaccination. By day 42, 50% of the horses ended with a L. canicola titer below 1:200, and 50% had a titer concentration at or above 1:200.

Conclusion

This study provided seroprevalence data that allowed comparisons to be made with previously published studies from different locations. In comparison, the Murray State Equine Center herd showed a higher seroprevalence for Leptospirosis in unvaccinated horses. Data from this project furthered the available information on seroprevalence of Leptospirosis in unvaccinated horses. The details on horses' response to the vaccination and booster provide data on how horses respond to the vaccine and how it affected their titers. A titer level that supports immunity was not determined, as it was not an objective of this study, but this data combined with further research may lead to a verified titer concentration needed to protect horses against Leptospirosis.

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Appendix A: IACUC Approval

MURRAY STATE UNIVERSITY
Institutional Animal Care and Use Committee

September 14, 2022

Dr. C.A. Shea Porr
Animal/Equine Science
Murray State University
Murray, KY 42071

Dear Dr. Porr:

The Murray State University Institutional Animal Care and Use Committee (IACUC) has approved your research protocol for the course titled, "Himmelsbaugh-Porr Lepto Titers in Horses."

The teaching protocol timeline is approved through June 2023. Please use the Animal Use Report (attached) to keep up-to-date information about the animals. At the termination of the protocol, you will need to complete the Conclusion Report (attached) and list final information concerning the animals.

If you have any questions, please contact me at (270) 809-3534.

Sincerely,

Kristi Stockdale

Kristi Stockdale
IACUC Coordinator

cc:
IACUC File