Review Article

Host Factors that Contribute to Equine Arteritis Virus Persistence in the Stallion: An Update

Udeni B. R. Balasuriya*, Sanjay Sarkar, Mariano Carossino, Yun Young Go^a, Lakshman Chelvarajan, R. Frank Cook, Alan. T. Loynachan^b, Peter J. Timoney, and Ernest Bailey

108 Maxwell H. Gluck Equine Research Center, and ^bUniversity of Kentucky Veterinary Diagnostic Laboratory, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546, USA

^aCurrent address: *Virus Research and Testing Group, Division of Drug Discovery Research, Korea Research Institute of Chemical Technology, Daejeon, South Korea*

*Corresponding author

Mailing address: 108 Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099

Phone: 859-218-1124; Fax: 859-257-8542; E-mail: ubalasuriya@uky.edu

Abstract: 248; Text: 2882 words; Figures: 5

Keywords: Equine arteritis virus, equine viral arteritis, EAV, EVA, persistent infection and host genetics

Abstract: Studies in our laboratory have shown that horses can be divided into two distinct groups based on whether or not they possess a subpopulation of CD3⁺ T lymphocytes susceptible to *in vitro* infection by equine arteritis virus (EAV). It has been also demonstrated that stallions with the CD3⁺ T lymphocyte susceptibility phenotype to *in vitro* EAV infection are at higher risk of becoming persistently infected carriers compared to those that lack this phenotype. Furthermore, experimental EAV infection of horses with either the *in vitro* CD3⁺ T cell susceptibility or resistance phenotype showed a significant difference between the two groups in terms of proinflammatory and immunomodulatory cytokine mRNA expression. A genome-wide association study (GWAS) demonstrated that these phenotypes are associated with the CXCL16 gene located in equine chromosome 11. We demonstrated that the membrane bound form of the protein encoded by EQCXCL16 function as a primary receptor molecule for EAV. Moreover, the secretory form of the CXCL16 protein is the ligand for the chemokine receptor CXCR6 that is predicted to be expressed on CD4⁺ and CD8⁺ T cells, NKT cells, and NK cells. Thus, equine CXCL16 and CXCR6 may be two major cellular proteins associated with the EAV carrier state in stallions. Finally, analysis of multiple tissues from EAV carrier stallions unequivocally confirmed the ampullae of the vas deferens as the primary site of EAV persistence with dual immunohistochemistry studies showing that EAV is localized in fibrocytes and mononuclear cells (T and B lymphocytes) but not in the glandular epithelium.

1. Introduction

Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease that is exclusively limited to members of the family *Equidae*, which includes horses, donkeys, mules, and zebras [1, 2]. While most natural EAV infections are clinically inapparent, relatively virulent field strains of EAV that can give rise to outbreaks of EVA periodically emerge around the world [3, 4]. The incubation period varies from 2 to 14 days (usually 6 to 8 days following venereal exposure), and the disease is characterized by fever (greater than 41°C); depression; leukopenia; excessive lacrimation; anorexia; conjunctivitis; rhinitis and nasal discharge; urticaria of the head, neck, and trunk; and edema, which is most pronounced over the eyes (supraorbital), the abdomen, prepuce, scrotum, mammary glands, and hind limbs [2]. Abortion is a frequent outcome in naïve pregnant mares, and generally occurs 10–30 days after exposure to EAV and at any time between 3 and 10 months of gestation [2, 3, 5-8]. Congenital infection in neonatal foals is characterized by severe fulminating interstitial pneumonia [9]. One to three--month-old foals can develop a progressive “pneumoenteric” syndrome leading to death frequently associated with secondary bacterial infections. A variable proportion of acutely infected stallions (10 to 70%) may become persistently infected and continuously shed the virus exclusively in their semen (carrier state) after clinical recovery from the acute phase of the infection (Figure 1) [10, 11].

The severity of the clinical signs exhibited by EAV-infected horses and the establishment of the carrier state are dependent on the interaction between the host, virus, and environmental factors (Figure 2). Viral factors (determinants) associated with the virulence, neutralization, and persistent infection in *in vitro* cell culture systems have been identified using virus reverse genetics and reviewed in several recent publications [12-14]. However, very little is known about the host factors involved in the establishment and long-term maintenance of EAV in the

reproductive tract of infected stallions. Persistence is a state of infection in which the host response is unable to eliminate the virus resulting in its continued replication with or without associated damage to the host tissues over time, which depends on the balance of host and viral factors and their interactions. In the case of EAV, this has almost certainly evolved to ensure the continued survival of this virus in equine populations. Therefore, in stark contrast to human immunodeficiency virus (HIV) [15, 16], persistent EAV infection in stallions does not result in overt clinical signs during chronic infection, and while infected animals are able to clear the virus from every other tissue in the body, the virus is able to persist in the reproductive tract of some stallions. Therefore, it can be argued that EAV has evolved a “masterpiece” mechanism whereby continual viral replication and immune evasion does not affect the ability of carrier stallions to breed. As a result, susceptible naïve mares can be infected via the venereal route leading to outbreaks of EVA characterized by respiratory disease and abortions. The mechanism(s) of establishment and maintenance of EAV persistent infection in the stallion’s reproductive tract is just beginning to be unveiled. The focus of this article is to provide an update on important recently identified host factors that may be associated with the establishment of the EAV carrier state in the stallion.

2. EAV persistent infection in the stallion:

The equine immune system can mount a strong immune response against many viral pathogens, resulting their successful elimination and resolution of infection [17-19]. Similarly, both natural and experimental EAV infection induces a solid and durable immune response that protects horses against subsequent infections [14, 20]. Both arms of the immune system (innate, and adaptive) are involved in providing protection against EAV [13, 14, 21, 22]. However, EAV

can evade the immune system to establish persistent infection only in certain stallions, which makes it a very unique example of a long-term natural carrier state in animals without evidence of clinical signs. The establishment and maintenance of EAV carrier state is influenced by sex and many other host factors, such as age, hormonal status, breed, immune status, and genetic background (Figure 1).

There are several important features that make the EAV carrier state different from persistence encountered in other viral diseases of horses, other animal species, and humans: (i). Persistent infection can be maintained in the reproductive tract of post-pubertal colts and stallions for a variable interval of time, from several months to life-long (Figure 2), (ii). It has been shown that persistent EAV infection in the stallion is testosterone dependent, (iii). There is no evidence of EAV causing persistent infection in mares, geldings, or foals demonstrating the dependence on specific male reproductive tract tissues and hormonal factors, (iv). EAV primarily persists in the ampullae of the vas deferens and not in immunologically privileged tissues such as the testes, (v). The virus persists in the male reproductive tract of carrier stallions despite the presence of high neutralizing antibody titers in the serum, (vi). Not all EAV infected stallions will become long-term carriers, and the establishment of the carrier state occurs at a higher frequency in some breeds (e.g. Standardbreds and Saddlebred) than in others (e.g. Thoroughbred) suggesting the existence of an underlining genetic element(s) predisposing to persistent infection, (vii). Semen quality is apparently normal in persistently infected stallions, despite high titers of virus shed in the semen [23]. The virus seems to exert little to no direct reproductive effect, as virus titers remain high long after semen quality returns to baseline levels following acute infection with EAV [23], and (viii). In the course of persistent infection, the virus undergoes rapid genetic evolution at a rate of approximately 10 nucleotide substitutions per

1000 bases per year [24]; this can lead to the emergence of novel genotypic and phenotypic variants. Collectively, these features suggest that persistent infection of EAV in the stallion's reproductive tract is both complex and multifactorial.

Transmission of EAV between horses occurs via either the respiratory or venereal route [2, 10, 11, 25-27]. Nevertheless, EAV persistent infection in the male reproductive tract provides an opportunity for the virus to be maintained during breeding seasons and transmitted to naïve mares at the time of natural service and/or artificial insemination; recently infected mares can in turn readily transmit the virus by the respiratory route to susceptible cohorts in close proximity including post-pubertal colts and stallions which may become persistently infected carriers [25]. The virus can also be transmitted to naïve recipient mares via embryo transfer from a donor mare inseminated with EAV-infective semen [28]. Movement of carrier stallions, contaminated frozen or chilled semen, and contaminated embryos have increased the spread of the virus nationally and internationally [28-30]. Carrier stallions solely constitute the natural reservoir of the virus, and thus, play a central and critical role in the epidemiology of EVA.

3. Host factors associated with the EAV carrier state in stallions:

It is clear that host genetic polymorphisms can affect viral pathogenesis in animals. Unfortunately, most host-pathogen interactions are complex and affected by multiple host genetic determinants making them difficult to study. Therefore, it is not surprising that until recently most emphasis was placed on the far simpler option of identifying just the viral factors affecting pathogenesis. However, the development of powerful genomic tools is rapidly changing this situation permitting investigators to elucidate the full effects of host genetics on disease outcome. The publication of a high-quality draft of the horse genome (*Equus caballus*),

has enabled the development of a rapid genetic screening tool based on single nucleotide polymorphisms (SNPs) [31]. These SNPs are single nucleotide base-pair variations between individual members of a species that occur at specific locations in the genome. As such, they can be used as a “roadmap” to identify the chromosomal regions associated with the hereditary traits such as susceptibility or resistance to a certain disease [32-35]. Assays to detect SNPs involve the use of allele-specific oligonucleotide probes bound to a solid matrix that is frequently termed a “chip” (e.g. Illumina Equine SNP50 chip, Illumina Inc., San Diego, CA). The availability of the equine SNP chip has provided an unparalleled opportunity to investigate genetic aspects of susceptibility of some stallions that become carriers following natural or experimental EAV infection.

In a previous study using dual-color flow cytometry, we demonstrated that the virulent Bucyrus strain of EAV (EAV VBS) can infect a small subpopulation of CD3⁺ T lymphocytes *in vitro* from some but not all horses [36], suggesting that horses can be divided into susceptible and resistant groups (Figure 3) based on this phenotypic trait [36]. The CD3⁺ T lymphocyte susceptible/resistant phenotype is not associated with age, prior exposure to EAV, or the presence of virus-specific antibodies but appeared to show an association with breed [37]. In fact, the susceptible/resistant phenotypic prevalence among four breeds was similar to EAV seroprevalence that has been reported previously (Figure 4) [38, 39]. The similarities in distribution between breed-associated seroprevalence, carrier status in stallions of different breeds and the CD3⁺ T lymphocyte susceptible trait strengthened our hypothesis that these traits might be linked by a single host genetic factor. This hypothesis was further supported by the finding that a subset of CD3⁺ T lymphocytes from long-term EAV carrier stallions was susceptible to *in vitro* EAV infection (Figure 5) [40]. In contrast, EAV seropositive naturally

infected stallions possessing the CD3⁺ T lymphocyte resistant phenotype did not become long-term carriers of the virus. Taken together, these observations clearly suggest a genetic association between the susceptibility/resistance phenotype of equine CD3⁺ T lymphocytes to *in vitro* EAV infection and the establishment of the carrier state. Subsequently, a genome-wide association study (GWAS) using the Illumina Equine SNP50 chip confirmed that both traits are associated with a region located on equine chromosome 11 (ECA11) between positions 49,572,804 and 49,643,932 [41]. Interestingly, the *in vitro* CD3⁺ T susceptible phenotype and the EAV long-term carrier state in the stallion were associated with four coding mutations in the EQCXCL16 gene that resulted in four amino acid changes in the amino-terminal ectodomain of the CXCL16 protein (a variant form of CXCL16) [42]. Its distribution was consistent with a dominant mode of inheritance. Moreover, we also identified a transmembrane form of EqCXCL16 possessing four amino acid changes that appear to play a major role in EAV host-cell entry processes, acting as one of the cellular receptor molecules for this virus [43]. The CXCL16 is expressed in monocytes and endothelial cells and therefore, it is tempting to suggest that this constitutes the mechanism underlying both CD3⁺ T lymphocyte susceptibility and EAV carrier status in stallions. However, the tissue distribution of EqCXCL16 in horses has not been determined. Consequently, further investigations are required before the mechanism(s) underlying CD3⁺ T lymphocyte susceptibility or the establishment and maintenance of EAV carrier status in stallions can be fully elucidated. Interestingly, there is also a secretory form of CXCL16, which is the ligand for the chemokine receptor CXCR6 a molecule that is predicted to be expressed on equine CD4⁺ and CD8⁺ T cells, NKT cells and NK cells. Thus, CXCR6 may also be involved in establishment and maintenance of EAV in stallions. In summary, the findings outlined above demonstrate that CD3⁺ T cell subpopulation susceptibility or resistance to viral

infection and the development of persistent EAV infection in the stallion reproductive tract is determined by CXCL16 located on chromosome ECA11 in association with its receptor CXCR6. However, the actual mechanism(s) by which these phenotypic traits are controlled by these proteins has not been determined.

4. Local response to EAV infection in the stallion reproductive tract:

Although EAV localization in the male reproductive tract has previously been identified, there is a lack of information on cellular localization of the virus [44, 45]. Some of our ongoing studies are focused on identifying the tissue and cellular localization sites of EAV during persistent infection in the reproductive tract of the stallion with the long-term goal of determining the underlying mechanism(s) by which EAV can establish and maintain persistent infection. Specifically, we have used archived tissues from long-term carrier stallions, stallions that were infected with EAV but had stopped shedding detectable virus in semen, and uninfected control animals [46]. The specific sites of EAV persistence in the reproductive tract were determined by virus isolation and single and dual immunohistochemistry (IHC). The ampulla was shown to be the tissue with the highest viral titer followed by the other accessory sex glands of carrier stallions. Within the ampullae, dual IHC demonstrated the presence of viral antigen in fibrocytes, lymphocytes (mainly CD3⁺ T lymphocytes and CD21⁺ B cells), and other mononuclear cells in the lamina propria and inflammatory infiltrates but not in glandular epithelium. The presence of inflammatory infiltrates in close proximity to EAV antigen expressing cells suggests that viral replication and persistence occurs despite the existence of ongoing active immune responses.

An unusual feature of EAV persistence in the reproductive tract of the stallion is that the virus does not exploit immunologically privileged tissues, such as the testes, and that viral replication occurs in the presence of active immune responses as evidenced by the presence of inflammatory infiltrates. At present it is not known why these apparently active immune response fails to eliminate the virus. Potential explanations include continual genetic evolution of EAV leading to the emergence of antigenic variants, a local immunosuppressive environment mediated by cytokines and hormones such as androgens, induction of peripheral tolerance via T-regulatory lymphocytes, or even immune exhaustion caused by continual antigenic stimulation resulting in effector T cells becoming progressively dysfunctional and less responsive. Such mechanisms are not mutually exclusive and could occur in combination, but further studies will be needed.

5. Other factors involved in EAV infection

Other host factors involved in EAV pathogenesis are poorly characterized. EAV infects alveolar macrophages (AM Φ) after respiratory infection of horses, but *in vitro* studies using cultured equine AM Φ confirmed that although these cells were susceptible to infection, they were refractory to productive replication of EAV [47]. In contrast, productive replication occurred following EAV infection of cultured blood-derived equine macrophages (BM Φ ; [47]. EAV infection of both equine AM Φ and BM Φ resulted in increased transcription of genes encoding proinflammatory mediators, including IL-1 β , IL-6, IL-8, and TNF- α , with the release of substantial quantities of TNF- α into the culture medium [47]. Furthermore, virulent and avirulent strains of EAV induced differential expression of TNF- α and other proinflammatory cytokines (IL-1b, IL-6, IL-8), and the

magnitude of the cytokine response of equine AM Φ and BM Φ to EAV infection was dependent upon the virulence of the infecting virus strain [47]. In addition, the expression of leukocyte adhesion molecules is upregulated following EAV infection. Recently, experimental inoculation with EAV into horses with *in vitro* CD3⁺ lymphocyte susceptibility or resistance showed a significant difference between the two groups of horses in terms of proinflammatory and immunomodulatory cytokine mRNA expression and evidence of increased clinical signs in horses possessing the *in vitro* CD3⁺ T lymphocyte resistant phenotype [48]. These studies provide direct evidence of a correlation between variation in host genotype and phenotypic differences in terms of the extent of viral replication, occurrence and severity of clinical signs, and cytokine gene expression that result from EAV infection in individual horses. However, the various cytokine gene expression profiles in the reproductive tissues (i.e. ampullae) of the carrier stallions have not been determined.

6. Conclusions:

Our studies have demonstrated that *in vitro* susceptibility of a subpopulation of equine CD3⁺ T lymphocytes to EAV infection correlates with the establishment of persistent infection in the reproductive tract of certain stallions. Furthermore, both of these phenotypic traits are strongly associated with the gene encoding CXCL16 (EqCXCL16) located on ECA11. The phenotype associated with CD3⁺ T lymphocyte susceptibility and persistence in stallions displays a classical dominant mode of inheritance. CXCL16 protein has scavenger receptor properties and can function as an entry receptor for EAV [43], however its distribution in cells of the reproductive tract has not been determined. It is possible that interactions between the secretory form of EqCXCL16 and its receptor molecule EqCXCR6 could have a role in both

CD3⁺ T lymphocyte susceptibility and the carrier status in stallions, but this has not yet been determined.

In addition to establishing the genetic basis we have unequivocally demonstrated that EAV primarily persists in mononuclear lineage cells (T and B lymphocytes) and fibrocytes in the ampullae of carrier stallions. Furthermore, EAV persistent infection is associated with a localized inflammatory response. However, it is not clear why this inflammatory response is unable to eliminate the virus from the reproductive tract. Therefore, more studies are needed, to further characterize both virological and immunological responses before the mechanism(s) of EAV persistence in the reproductive tract of stallions can be completely understood.

It is anticipated that there will be practical benefits from this work such as development of a simple genetic test to identify stallions that are most at risk of becoming long term carriers following exposure to EAV. In addition, once the role of EqCXCL16 is fully elucidated the development of novel antiviral therapies to clear EAV from the stallion's reproductive tract will be feasible.

7. Acknowledgement:

This work was supported by Agriculture and Food Research Initiative competitive grant no. 2013-68004-20360 from the USDA National Institute of Food and Agriculture, the Hildegard Rosa Shapiro Endowed Equine Research Fund endowment fund at the Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, the Morris Animal Foundation, Denver, CO, Maxwell H. Gluck Equine Research Center intramural research grant program and the Frederick Van Lennep Chair endowment fund at the Maxwell H. Gluck Equine Research Center, University of Kentucky.

References:

- [1] Stadejek T, Mittelholzer C, Oleksiewicz MB, Paweska J, Belak S. Highly diverse type of equine arteritis virus (EAV) from the semen of a South African donkey: short communication. *Acta Vet Hung.* 2006;54:263-70.
- [2] Timoney PJ, McCollum WH. Equine viral arteritis. *Vet Clin North Am Equine Pract.* 1993;9:295-309.
- [3] McCollum WH, Timoney, P. J. Lee Jr, J. W., Habacker, P. L., Balasuriya, U. B. R., and MacLachlan, N. J. Features of an outbreak of equine viral arteritis on a breeding farm associated with abortion and fatal interstitial pneumonia in neonatal foals. In: Wernery U, Wade, J. F., Mumford, J. A., Kaaden, O. -R., editor. *Proceeding of the 8th International Conference on Equine Infectious Diseases.* Dubai: R & W Publications (Newmarket) Limited; 1998. p. 559-60.
- [4] McCollum WH, Timoney PJ. Experimental observations on the virulence of isolates of equine arteritis virus. In: Wernery U, Wade, J. F., Mumford, J. A., Kaaden, O. -R., editor. *Proceedings of the 8th International Conference Equine Infectious Diseases.* Dubai: R & W Publications (Newmarket) Limited; 1998. p. 558-9.
- [5] Timoney PJ, Fallon, L., Shuck, K., McCollum, W. H., Zhang, J., and William, N. . The outcome of vaccinating five pregnant mares with a commercial equine viral arteritis vaccine. *Equine Veterinary Education.* 2007;19:6.
- [6] Timoney PJ, Klingeborn B, Lucas MH. A perspective on equine viral arteritis (infectious arteritis of horses). *Rev Sci Tech.* 1996;15:1203-8.
- [7] Timoney P. J aMWH. Equine viral arteritis: Current clinical and economic significance *Proc Am Assoc Eq Pract* 1991. p. 403-9.
- [8] Timoney PJ. Clinical, virological, and epidemiological features of the 1984 outbreak of equine viral arteritis in the Thoroughbred population in Kentucky, USA. *Proceedings of the Grayson Foundation International Conference of Thoroughbred Breeders Organizations.* Dromoland Castle, Ireland 1984. p. 24-33.
- [9] Vaala WE, Hamir AN, Dubovi EJ, Timoney PJ, Ruiz B. Fatal, congenitally acquired infection with equine arteritis virus in a neonatal thoroughbred. *Equine Vet J.* 1992;24:155-8.
- [10] Timoney PJ, McCollum WH, Murphy TW, Roberts AW, Willard JG, Carswell GD. The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of virus transmission. *J Reprod Fertil Suppl.* 1987;35:95-102.
- [11] Timoney PJ, McCollum WH, Roberts AW, Murphy TW. Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. *Res Vet Sci.* 1986;41:279-80.
- [12] Balasuriya UB, Zhang J, Go YY, MacLachlan NJ. Experiences with infectious cDNA clones of equine arteritis virus: lessons learned and insights gained. *Virology.* 2014;462-463:388-403.
- [13] Balasuriya UB. Equine viral arteritis. *Vet Clin North Am Equine Pract.* 2014;30:543-60.
- [14] Balasuriya UB, Go YY, MacLachlan NJ. Equine arteritis virus. *Vet Microbiol.* 2013;167:93-122.
- [15] Levy JA. Pathogenesis of human immunodeficiency virus infection. *Ann N Y Acad Sci.* 1989;567:58-68.
- [16] Mims CA. Pathogenesis of infection with the human immunodeficiency virus--a personal view. *J Infect.* 1988;17:221-9.
- [17] Horohov DW. The equine immune responses to infectious and allergic disease: a model for humans? *Mol Immunol.* 2015;66:89-96.

- [18] Horohov DW. Equine T-cell cytokines. Protection and pathology. *Vet Clin North Am Equine Pract.* 2000;16:1-14.
- [19] Horohov DW, Lunn DP, Townsend HG, Wilson D. Equine vaccination. *J Vet Intern Med.* 2000;14:221-2.
- [20] Balasuriya UB, MacLachlan NJ. The immune response to equine arteritis virus: potential lessons for other arteriviruses. *Vet Immunol Immunopathol.* 2004;102:107-29.
- [21] Go YY, Li Y, Chen Z, Han M, Yoo D, Fang Y, et al. Equine arteritis virus does not induce interferon production in equine endothelial cells: identification of nonstructural protein 1 as a main interferon antagonist. *BioMed research international.* 2014;2014:420658.
- [22] MacLachlan NJ, Balasuriya UB, Hedges JF, Schweidler TM, McCollum WH, Timoney PJ, et al. Serologic response of horses to the structural proteins of equine arteritis virus. *J Vet Diagn Invest.* 1998;10:229-36.
- [23] Campos JR, Breheny P, Araujo RR, Troedsson MH, Squires EL, Timoney PJ, et al. Semen quality of stallions challenged with the Kentucky 84 strain of equine arteritis virus. *Theriogenology.* 2014;82:1068-79.
- [24] Hedges JF, Balasuriya UB, Timoney PJ, McCollum WH, MacLachlan NJ. Genetic divergence with emergence of novel phenotypic variants of equine arteritis virus during persistent infection of stallions. *J Virol.* 1999;73:3672-81.
- [25] Cole JR, Hall RF, Gosser HS, Hendricks JB, Pursell AR, Senne DA, et al. Transmissibility and abortogenic effect of equine viral arteritis in mares. *J Am Vet Med Assoc.* 1986;189:769-71.
- [26] Doll ER, Knappenberger RE, Bryans JT. An outbreak of abortion caused by the equine arteritis virus. *Cornell Vet.* 1957;47:69-75.
- [27] McCollum WH, Prickett ME, Bryans JT. Temporal distribution of equine arteritis virus in respiratory mucosa, tissues and body fluids of horses infected by inhalation. *Res Vet Sci.* 1971;12:459-64.
- [28] Broaddus CC, Balasuriya UB, Timoney PJ, White JL, Makloski C, Torrisi K, et al. Infection of embryos following insemination of donor mares with equine arteritis virus infective semen. *Theriogenology.* 2011;76:47-60.
- [29] Broaddus CC, Balasuriya UB, White JL, Timoney PJ, Funk RA, Holyoak GR. Evaluation of the safety of vaccinating mares against equine viral arteritis during mid or late gestation or during the immediate postpartum period. *J Am Vet Med Assoc.* 2011;238:741-50.
- [30] Zhang J, Timoney PJ, Shuck KM, Seoul G, Go YY, Lu Z, et al. Molecular epidemiology and genetic characterization of equine arteritis virus isolates associated with the 2006-2007 multi-state disease occurrence in the USA. *J Gen Virol.* 2010;91:2286-301.
- [31] Wade CM, Giulotto E, Sigurdsson S, Zoli M, Gnerre S, Imsland F, et al. Genome sequence, comparative analysis, and population genetics of the domestic horse. *Science (New York, NY).* 2009;326:865-7.
- [32] Chowdhary BP, Paria N, Raudsepp T. Potential applications of equine genomics in dissecting diseases and fertility. *Animal reproduction science.* 2008;107:208-18.
- [33] Davila S, Hibberd ML. Genome-wide association studies are coming for human infectious diseases. *Genome Med.* 2009;1:19.
- [34] Hill AV. Aspects of genetic susceptibility to human infectious diseases. *Annu Rev Genet.* 2006;40:469-86.
- [35] Swinburne J. Inherited disease in the horse: mapping complex disease variants is on the horizon. *Vet J.* 2009;179:317-8.

- [36] Go YY, Zhang J, Timoney PJ, Cook RF, Horohov DW, Balasuriya UB. Complex interactions between the major and minor envelope proteins of equine arteritis virus determine its tropism for equine CD3+ T lymphocytes and CD14+ monocytes. *J Virol*. 2010;84:4898-911.
- [37] Go YY. Molecular and genomic approaches to understanding host-virus interactions in shaping the outcome of equine arteritis virus infection. Lexington: University of Kentucky; 2011.
- [38] Hullinger PJ, Gardner IA, Hietala SK, Ferraro GL, MacLachlan NJ. Seroprevalence of antibodies against equine arteritis virus in horses residing in the United States and imported horses. *J Am Vet Med Assoc*. 2001;219:946-9.
- [39] McCollum WH, Bryans JT. Serological identification of infection by equine arteritis virus in horses of several countries. In: Gerber JTBaH, editor. *Proceedings of the 3rd International Conference on Equine Infectious Diseases*. Paris: S. Karger, Basel; 1973. p. 256-63.
- [40] Go YY, Bailey E, Timoney PJ, Shuck KM, Balasuriya UB. Evidence that in vitro susceptibility of CD3+ T lymphocytes to equine arteritis virus infection reflects genetic predisposition of naturally infected stallions to become carriers of the virus. *J Virol*. 2012;86:12407-10.
- [41] Go YY, Bailey E, Cook FR, Coleman SJ, Macleod JN, Chen KC, et al. Genome-wide association study among four horse breeds identifies a common haplotype associated with in vitro CD3+ T cell susceptibility/resistance to equine arteritis virus infection. *J Virol*. 2011;85:13174-84.
- [42] Sarkar S, Bailey, E., Go, Y. Y., Kalbfleisch, T., Chelvarajan, L., Cook, F., Eberth, J., Bhowmik, M., Artushin, S., Timoney, P. J., and Balasuriya, U. B. R. Variants of CXCL16 gene is associated with in vitro CD3+ T cell susceptibility to equine arteritis virus infection. *Nature Genetics*. 2016.
- [43] Sarkar S, Chelvarajan L, Go YY, Cook F, Artiushin S, Mondal S, et al. Equine Arteritis Virus Uses Equine CXCL16 as an Entry Receptor. *J Virol*. 2016;90:3366-84.
- [44] Neu SM, Timoney, P. J., and Lowry, S. R. Changes in semen quality following experimental equine arteritis virus infection in the stallion. *Theriogenology*. 1992;37:407-31.
- [45] Little TV, Holyoak, G. R., McCollum, W. H., and Timoney, P. J. Output of equine arteritis virus from persistently infected stallions is testosterone dependent. In: Plowright P. R, D., Wade, J. F., editor. *Proceedings of the 6th international conference on equine infectious diseases*. Cambridge: R & W Publications, Newmarket, England; 1991. p. 225-9.
- [46] Carossino M, Loynachan, A. T., Campos, J. R., Bora, N., Canisso, I. F., Go, Y. Y., Troedsson, M. H., , Cook, R. F., Swerczek, T., Timoney, P. J., Squires, E. L., Del Piero, F., Bailey, E., and Balasuriya, U. B. R. Identification of Equine Arteritis Virus Host Cell Tropism in the Reproductive Tract of Persistently Infected Stallions. *PLoS Pathogen*. 2016.
- [47] Moore BD, Balasuriya UB, Watson JL, Bosio CM, MacKay RJ, MacLachlan NJ. Virulent and avirulent strains of equine arteritis virus induce different quantities of TNF-alpha and other proinflammatory cytokines in alveolar and blood-derived equine macrophages. *Virology*. 2003;314:662-70.
- [48] Go YY, Cook RF, Fulgencio JQ, Campos JR, Henney P, Timoney PJ, et al. Assessment of correlation between in vitro CD3(+) T cell susceptibility to EAV infection and clinical outcome following experimental infection. *Veterinary microbiology*. 2011.

Figure legends:

Figure 1. Schematic representation of the outcome of EAV infection in horses. Stallions become persistently infected carriers of the virus. The carrier state can be short or long term. Modified from Balasuriya *et al.* (2015) with permission from the Vet Clin North Am Equine Prac.

Figure 2. The interaction between the infectious agent, host factors and environmental factors determines the outcome of EAV infection in horses. These interactions are complex, multifactorial, and mostly determined by viral and host factors, which include host genetics.

Figure 3. Peripheral blood mononuclear cells from over 800 horses representing four horse breeds (Thoroughbred [TB], Standardbred [STB], American Saddlebred [ASB] and Quarter Horses [QH]) were infected *in vitro* with the EAV virulent Bucyrus strain (VBS) and subjected to dual color flow cytometry (the cells were stained 36 hours post infection with antibodies against the CD3 surface marker and the viral nonstructural protein 1 [nsp1]). *In vitro* susceptibility of CD3⁺ T lymphocytes to EAV virulent Bucyrus strain (VBS) segregates horses into two distinct groups (Group A and Group B). In Group A, CD3⁺ T lymphocytes are susceptible to VBS infection while those from Group B horses are resistant. Thus, host genetic differences may be responsible for CD3⁺ T lymphocytes susceptibility/resistance to infection with EAV VBS strain.

Figure 4. Association between EAV seroprevalence among horse breeds and the *in vitro* T lymphocyte susceptible/resistant phenotype. The percentage of susceptible (red) and resistant

(blue) phenotypes for each breed is indicated in the bar graph. Seroprevalence of each breed is indicated on the right hand box. TB, Thoroughbreds (n=137); STB, Standardbreds (n=60); ASB, American Saddlebreds (n=60); QH, Quarter horses (n=53).

Figure 5. Dual-color flow cytometry analysis of T lymphocytes from carrier and seropositive non-carrier stallions following *in vitro* infection with EAV. (A) Carrier status was confirmed by repeatedly isolating EAV from semen over an extended period of time. (B) Non-carrier stallions were uninfected but seropositive to EAV following natural infection, not vaccination. Lymphocytes from each stallion that were labeled with both anti-CD3 and viral anti-nsp1 (12A4) MAbs are shown in the upper right quadrant of each dot plot. Note significant double labeling of lymphocytes from the carrier stallion but not the non-carrier stallion for both CD3 and EAV after *in vitro* infection. Modified from Go et al. (2012) with permission from the J Virol.

Figure 1

Figure 2

N= >800

(TB, STB, ASB, QH etc.)

**EAV Infected
CD3⁺ T cell
Subpopulation**

**CD3⁺ T cell
Susceptible
Phenotype**

**CD3⁺ T cell
Resistant
Phenotype**

Figure 3

Figure 4

A. Carrier Stallions

B. Non-carrier Stallions