PEER REVIEWED

Diverse outcomes of bovine viral diarrhea virus (BVDV) infections in a herd naturally infected during pregnancy–a case study

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Abstract

A beef producer purchased pregnant Angus crossbred cows with nursing calves. The purchased cattle, their nursing calves, and subsequent born calves were not initially tested for bovine viral diarrhea virus (BVDV), nor was there a history of vaccination. Bovine viral diarrhea virus subtype 2a was later isolated from an aborted bovine fetus estimated to be 6.5 months gestational age. The fetus had multiple congenital malformations including arthrogryposis, kyphosis, scoliosis, polydactylism, and cardiac overriding aorta. Testing by immunohistochemistry and virus isolation resulted in the detection of potentially persistently infected cattle, including a yearling and a calf born during the same calving season as the aborted fetus. Viruses isolated from the malformed fetus, the yearling, and the calf born during the same calving season were identical. The malformations observed in the aborted fetus were similar to arthrogryposis multiplexa (AM) and contractural arachnodactyly (CA), diseases associated with genetic defects in the Angus breed. The fetus was tested and found negative for the genetic defect linked with AM and CA. This case illustrates that suspect malformations should also be tested for BVDV, and underscores the potential for disease after failed or inadequate biosecurity.

Key words: BVDV, PI, abortion, fetal malformation

Résumé

Un producteur de bovins de boucherie a acheté des vaches gestantes Angus de race croisée avec des veaux allaitants. Les vaches achetées, les veaux allaitants et les veaux nés subséquemment n'avaient pas été testés initialement pour le BVDV et il n'y avait pas eu de vaccination au préalable. Le virus de la diarrhée virale bovine de sous-type 2a (BVDV2a) a été isolé plus tard d'un fœtus bovin avorté dont l'âge de gestation a été estimé à 6.5 mois. Le fœtus comportait de multiples malformations congénitales incluant l'arthrogrypose, la cyphose, la scoliose, la polydactylie et la malposition de l'aorte. Des tests immunohistochimiques et d'isolement du virus ont mené à la détection d'immunotolérance potentielle chez certains bovins, incluant un jeune de l'année et un veau né durant la même saison de vêlage que le fœtus avorté. Les virus isolés du fœtus malformé, du jeune de l'année et du veau né dans la même saison de vêlage étaient identiques. Les malformations observées chez le fœtus avorté étaient compatibles avec l'arthrogrypose multiple et l'arachnodactylie avec contractures, des maladies associées à des défauts génétiques chez la race Angus. Le fœtus a été testé pour ces deux défauts génétiques mais sans résultat positif. Ce cas démontre l'intérêt de tester les animaux avec malformations suspectes pour le BVDV et souligne le potentiel de maladie lorsque les mesures de biosécurité sont manquantes ou inadéquates.

Introduction

Bovine viral diarrhea viruses (BVDV) consist of 2 species, BVDV1 and 2, and are members of the family *Flaviviridae*, genus *Pestivirus*.[3] The principal subtypes in the United States are BVDV1a, BVDV1b, and BVDV2a.[3] Infections with BVDV are associated with several clinical presentations, in combination or singly, and include acute/transient, respiratory, digestive tract, thrombocytopenia/hemorrhagic, mucosal disease, repro-

ductive tract/fetal diseases, and persistent infection (PI) of calves resulting from in utero infections.[3] The BVDV are noteworthy for their immunosuppressive properties which impact multiple cellular and organ systems of the bovine.[3]

The purpose of this report is to explore the role of BVDV in a clinical presentation of fetal malformations. The report describes the occurrence of BVDV infection in an aborted fetus with malformations similar to arthrogryposis multiplexa (AM) and contractural arachnodactyly (CD), diseases with a genetic base in the Angus breed.

Herd History

In December 2010 a beef producer purchased 2 cows that were pregnant and 2 nursing calves (Table 1). The cows and calves were not tested for BVDV at time of purchase, and subsequent calves from the pregnancy were not tested for BVDV until later. One cow delivered a calf in April of 2011 (#52), and the calf was not tested for BVDV until 2012. Another 11 cow-calf pairs presumed not pregnant, with unknown BVDV history, were purchased in March 2011 and commingled with the cattle purchased in 2010.

Among the cows purchased in March 2011, cow #33 aborted on January 03, 2012 and cow #50 aborted a fetus on February 03, 2012 with suspect developmental anomalies. Cow #46 also aborted in 2012 on an undetermined date. The fetus from cow #50 was submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for necropsy because of the congenital defects. Aborted fetuses from cows #33 and #46 were not submitted for testing.

A breeding bull was purchased in June 2011, and later tested for BVDV by skin test (ear notch) by immunohistochemistry (IHC) at the OADDL.

Diagnostics

The fetus from cow #50 was approximately 6.5 months gestational age, consistent with breeding by the bull purchased in June 2011. Necropsy examination of the fetus confirmed multiple congenital anomalies

Table 1. Timeline for cattle addition to the herd and BVDV testing results.

Year	Month	Animals	IHC test results
2010	December	Two cows purchased at sale barn. Pregnant and with nursing calves. #16 and #31	Cows and calves not tested.
2011	January-February		
	March	Eleven cows purchased at sale barn and all with nursing calves, and presumed open. Commingled with resident cows #31 and #16 and their calves.	Cows and calves not tested.
	April	#31 cow delivered calf #52 and calf remained in herd until 2012. #16 had calf in spring 2011 (#Y26).	
	May		
	June	Bull purchased	IHC test negative
	July-December	Animals commingled	
2012	January	#33 cow from March 2011 aborted Jan 3, and #46 cow from March 2011 believed to have aborted fetus (date unknown). Fetuses not collected.	Cows #33 and #46 aborted and were IHC test-negative and sold.
	February	#50 cow from March 2011 aborted on Feb 5, and fetus sent to OADDL (OK 0375). Two cows purchased in Dec 2010, 11 cows purchased in March 2011, 4 calves born in 2012, and the #52 calf (yearling born to cow #31 in April 2011) were tested by BVDV IHC.	OK 0375 fetus with gross anomalies and BVDV-positive in tissues. All 13 cows were BVDV IHC-negative, as were 4 calves born in 2012. The yearling #52 was BVDV IHC-positive and BVDV2a was isolated.
	March-May		
	June	Six calves born in 2012 were tested with BVDV IHC.	Five calves were BVDV IHC-negative. One calf #5 born in 2012 was BVDV IHC-positive and BVDV2a was isolated.

including marked arthrogryposis, marked kyphosis, scoliosis, polydactylism, and cardiac overriding aorta. All 4 limbs had marked arthrogryposis, similar to those associated with genetic defects in the Angus breed resulting in AM and CA.[11] Tissues were collected for further testing, which included screening for BVDV.

Fresh tissue sections of liver and kidney from the malformed fetus were positive by fluorescent antibody testing (FAT) for BVDV antigen, whereas the lung was FAT negative.[6] Tissue homogenates from the liver, kidney, and lung were positive for BVDV2 by gel-based polymerase chain reaction.[4] Tissue homogenates were inoculated onto MDBK cultures, and BVDV isolates were obtained from liver, kidney, and lung.[7] The BVDV isolates were tested for subtype status using the sequences of the 5'-UTR, and were found to be BVDV2a.[5] The dam of the malformed fetus was negative for BVDV by skin-test IHC.[7] Subsequently, 13 cows with 5 calves born in 2012, and a yearling (#52) born to 1 of the cows purchased in December 2010, were tested for BVDV by IHC. Yearling calf #52 was positive for BVDV, and the remaining animals were negative. Serum obtained from animal #52 was inoculated onto MDBK monolayers, and BVDV2a was isolated (OK 52). This isolate was genetically identical to the BVDV2a isolated from the malformed fetus (OK O375). Subsequently, 6 more calves born in 2012 were tested for BVDV, and 1 (OK 5) was IHC positive on the skin-test tissue and virus isolation. The BVDV2a isolated was identical to the OK 0375 and OK 52 isolates (Figure 1).

Fetal kidney tissues from the malformed fetus (OK 0375) were submitted for genetics testing[a], and were negative for AM and CA.

The herd bull purchased in 2011 was negative when tested for BVDV by IHC.

Discussion

This case illustrates multiple outcomes resulting from BVDV fetal infections, including recovery of virus from tissues of a fetus with congenital malformations and from cattle presumed to be PI with no apparent clinical disease. Cattle infected with BVDV and/or initially positive by IHC were not retested. Sequence identity provides evidence that the 3 BVDV2a strains were identical. It is likely that introduction of the virus into the herd began during the pregnancy of the dam of yearling calf (OK 52), and that the original virus could have transiently infected the dam of the calf infected with BVDV (OK 5), as well as the cow which gave birth to the malformed fetus (OK 0375). While there were other abortions in this outbreak, no additional fetuses were available for testing. Those abortions may have resulted from BVDV exposure as abortions and malformed calves have been reported in other herd outbreaks. Although as in the current case, no aborted fetuses were tested for BVDV.[9] A recent report described abortions, premature births, and congenital anomalies due to fetal infection with BVDV1b.[1] In that study, fetal tissues of malformed fetuses were positive by FAT, and BVDV was isolated from fetal tissues.

Fetal infections with BVDV have varied outcomes with persistent infections, and include congenital malformations such as hydrocephalus, hydranencephaly, cerebellar hypoplasia, growth retardation, and mandibular brachygnathism.[2] Ocular lesions, along with CNS malformations, were reported following experimental infection of the bovine fetus.[10] The reasons for these varied outcomes following fetal infection with BVDV are not completely defined. Some variation, but not all, is due to gestational age of the fetus at time of infection. It is hypothesized that all fetal exposures prior to 125 days gestation result in PI. While this hypothesis is consistent with field and experimental observations to date, it has not been tested under controlled conditions. However, the development of other congenital defects do not appear to develop consistently, resulting in groups of calves exposed at approximately the same gestational stage displaying a range of congenital malformations, from clinically inapparent to severe.[1,8] This variation in outcome is illustrated in this report, with both a fetus with malformations and clinically normal calves presumed to be BVDV-PI born during the same calving season. No other fetal malformations in the herd were reported.

Calving seasons often vary due to the length of time cows are exposed to a bull, which may be as short as 30 to 60 days, or even continuous in some management systems. It is not known with certainty whether BVDV might cause abnormal fetal development similar to AM or CA. However, this case does suggest that infection with BVDV might be associated with gross lesions suggestive of AM and CA in a deformed calf.

Conclusions

This cases illustrates several issues for the clinician and diagnostician: 1) lesions due to genetic anomalies, infection, and toxin-based etiologies may result in similar presentations, and a complete workup should include tests for all 3 potential causes; 2) results of BVDV fetal infections in the same outbreak may vary based on fetal gestational age and rate of exposure (as illustrated in this case) with abortion, anomalies, and potential PI calves all possible; and 3) results of disease outbreaks can be traced to biosecurity failures. In the case reported here, biosecurity protocols were not followed. Further, the need for a biosecurity plan was not recognized by the owner. Unfortunately, veterinarians often become involved only during or after an outbreak occurs. Isolation of newly purchased cattle, and testing of

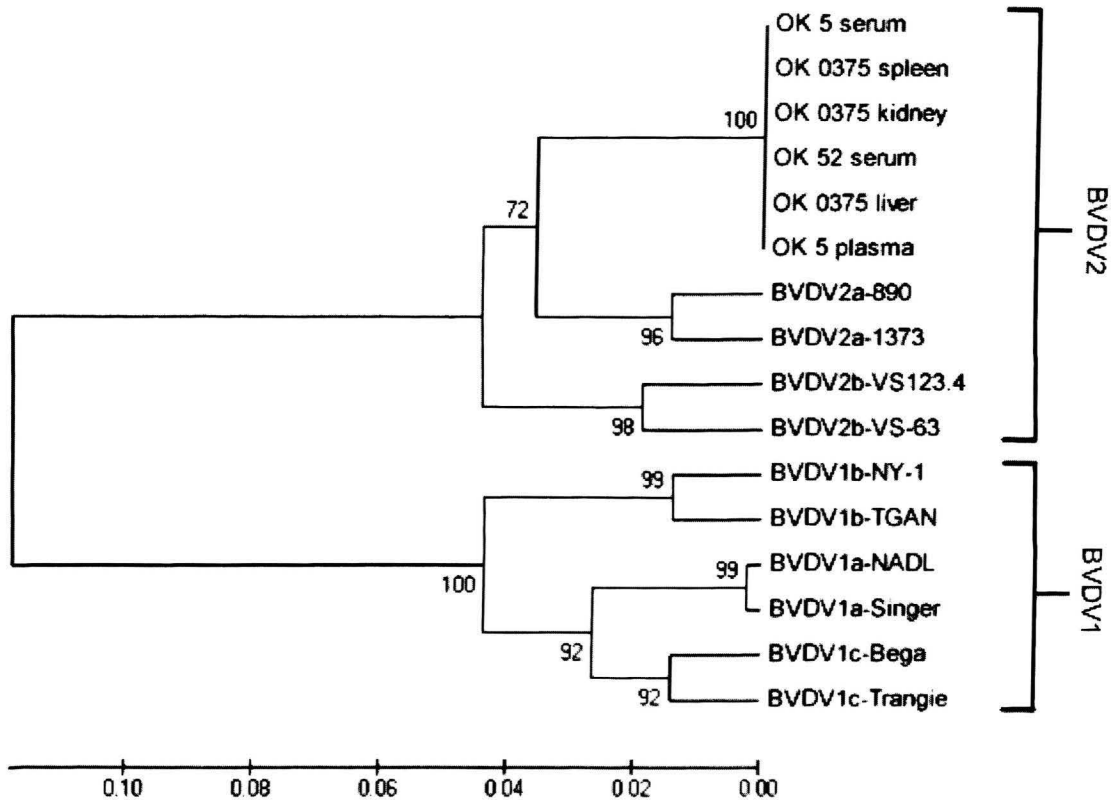

Figure 1. Bovine viral diarrhea virus phylogenetic tree demonstrating identity of BVDV2a strains in the outbreak.

purchased animals and the calves of purchased pregnant cows, would have precluded this outbreak. Reports of cases such as this offer educational opportunities where clinicians and diagnosticians can stress the importance of biosecurity programs to owners/producers.

Endnote

[a]AgriGenomics, Inc., Mansfield, IL

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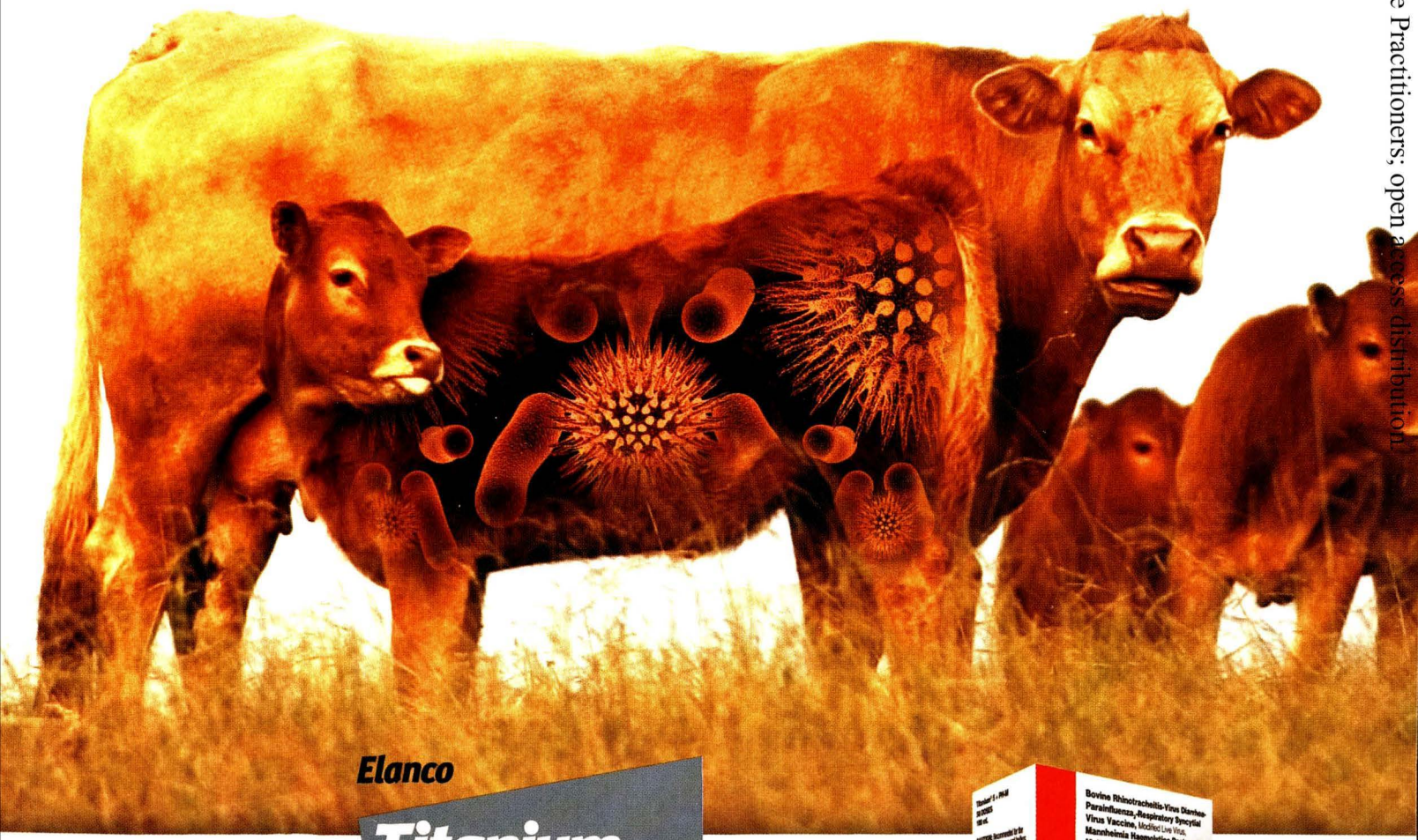

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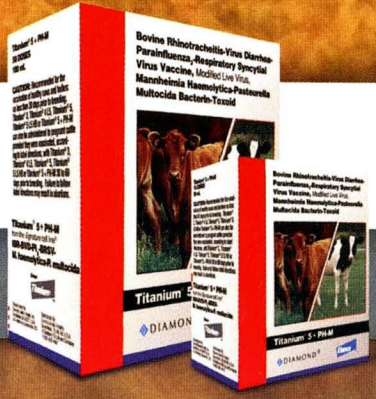

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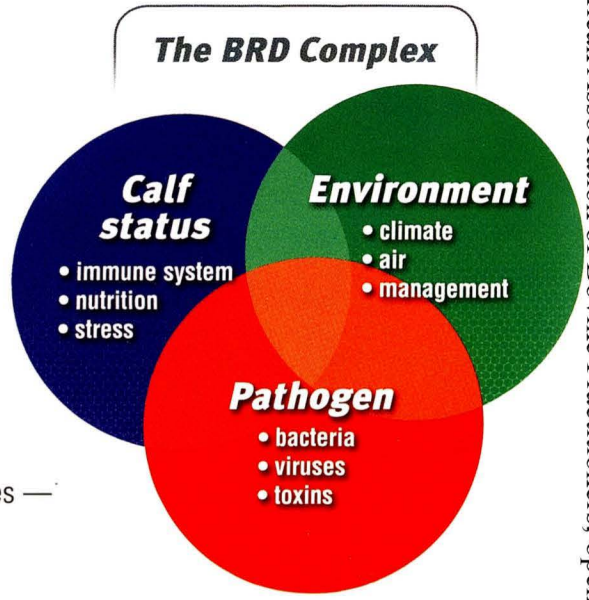
Titanium 5 + PH-M delivers a broad immune response against the bacteria and viruses most associated with bovine respiratory disease (BRD) in a single vaccine for cattle as young as 2 months of age.[1-4]

Managing the BRD complex — enhance immunity; reduce exposure to disease

The many factors affecting a calf's ability to contract BRD can be grouped into three categories that interact with one another: the infectious pathogens, the environment and the status of the calf. While some of these factors are within our control, many are not.

That's why your veterinarian uses best management practices and preventive measures, including vaccinations, to enhance the animal's immune system. Disease prevention can best be described as a two-pronged approach:

- Increase the animal's ability to overcome disease challenges through vaccination, which builds a stronger immune system
- Lower the level of exposure to disease through best management practices — this is especially critical during stressful events (e.g., weaning, transporting, commingling)



Viruses and bacteria — a powerful one-two punch on the immune system

While respiratory viruses can cause BRD on their own, they also can compromise the immune system that normally protects cattle against bacteria, allowing bacteria to attack their host and cause severe cases of BRD.

- When cattle are exposed to respiratory viruses — such as bovine viral diarrhea (BVD), parainfluenza$_3$ (PI$_3$), infectious bovine rhinotracheitis (IBR) and bovine respiratory syncytial virus (BRSV) — their immune system can be weakened
- Once the immune system is compromised, bacteria including *Mannheimia haemolytica* and *Pasteurella multocida* can more easily go deeper into the respiratory tract where they cause disease
- When bacterial pathogens reach the lungs, they are a major cause of severe BRD, causing increased morbidity, mortality, and labor and treatment costs

BRD is still your No. 1 profit robber

- BRD accounts for 75 percent of feedlot morbidity, and 50 to 75 percent of mortality, costing the industry an estimated $800 to $900 million annually[5-8]
- BRD-related factors also contribute to a reduction in average daily gain by as much as 0.3 to 0.5 lbs[9,10]
- And what about subclinical cases? One study showed that 68 percent of untreated calves had pulmonary lesions at slaughter, demonstrating that a significant number of animals never diagnosed with BRD do, in fact, suffer from some degree of respiratory disease[11]

Helping your cattle overcome the one-two punch of viruses and bacteria requires a vaccine solution that provides a broad immune response against the bacteria and viruses most associated with BRD.

Titanium 5 + PH-M — the convenience and flexibility of a single vaccine that protects against BRD-causing viruses and bacteria

Protects against the two bacteria most associated with BRD:[1]

- ***Mannheimia haemolytica*** — the bacterium most frequently isolated from lungs of cattle with BRD
- ***Pasteurella multocida*** — an important cause of bacterial pneumonia

Also protects against five important virus strains:[2,3]

- **BVD, types 1 and 2**
- **IBR**
- **PI_3**
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Approved for use in cattle 2 months of age and older, Titanium 5 + PH-M is flexible enough to be incorporated into virtually any vaccine protocol. Producers should work with their veterinarian to determine the best way to incorporate Titanium 5 + PH-M. As a general guide, it fits well in branding and/or preconditioning vaccine protocols, as well as in arrival programs for stocker and feedyard operations.

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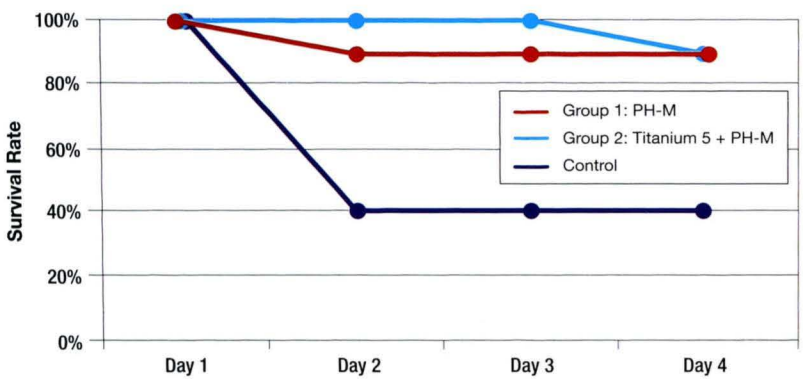
- Reduced lung lesions* by 82 percent ($P < 0.01$)
- Reduced clinical signs of infection significantly ($P < 0.01$)

*Mitigated fraction: the effect of a vaccine in reducing severity of disease.

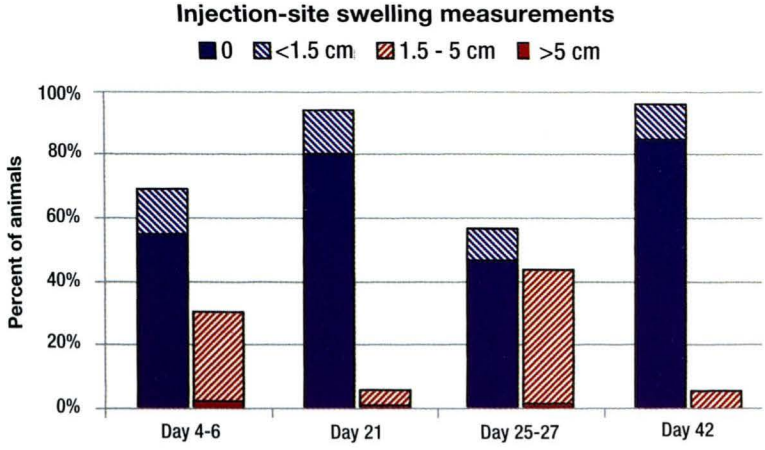
Figure 1. *M. Haemolytica* Challenge Study Survival Rate[1]



Studies also showed Titanium 5 + PH-M:

- Provided the same viral protection (BVD, types 1 and 2, IBR, PI_3, BRSV) as Titanium 5[2,3]
- Protected against severe clinical signs of BRD[1-3]
- Resulted in no adverse events beyond minimal injection-site swelling[4]

Figure 2. Titanium 5 + PH-M Safety Study Injection-Site Evaluation[4]



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