Pfizer Animal Health
Technical Bulletin

July 2004

CattleMaster®GOLD™: Inactivated BVDV fractions aid in the prevention of persistently infected calves

PfizerAnimal Health
Pfizer Inc
New York, NY 10017

Key Points

- The ability of prebreeding vaccination with *CattleMaster®GOLD™* to aid in the prevention of calves persistently infected with BVDV Types 1 and 2 was evaluated in two efficacy studies.[1,2]
- Following BVDV challenge, *CattleMaster GOLD* vaccinates showed a significant reduction in both the frequency and duration of viremia.
- Prebreeding vaccination of BVDV-seronegative cows and heifers with *CattleMaster GOLD* protected 100% of their calves against becoming persistently infected following virulent challenge with BVDV Type 1 or BVDV Type 2.

Of primary economic importance to cow-calf and dairy producers is preventing the birth of calves persistently infected (PI) with bovine viral diarrhea virus (BVDV). In given populations, approximately 0.4% to 1.7% of cattle are persistently infected.[3,4] Screening of 75 herds selected randomly from veterinary clinic client listings in five states in 1996 showed that 2.7% of the herds had confirmed or probable PI BVDV calves.[5] While relatively few in number, PI calves are a constant threat to herd health and profitability because they typically shed BVDV on a lifelong basis—often in large amounts—and are the principal means by which BVD herd infection and enzootic disease are perpetuated.[6] While many PI calves do not survive beyond 6 to 12 months of age, some PI calves appear healthy, reach maturity, and continue to shed virus to herdmates. The presence of PI calves makes it difficult for susceptible calves to escape infection when their passive immunity wanes, usually between 3 and 8 months of age.[3] The mechanism of transplacental transfer of BVDV is unknown; however, it has been demonstrated that even a small amount of virus is sufficient to cause development of PI calves immunotolerant to BVDV. In the estimation of some, true prevention and control of BVD presupposes elimination of PI cattle.[7]

CattleMaster GOLD is the latest innovation in bovine respiratory and reproductive vaccinology from Pfizer Animal Health. It represents a significant advance in BVD control. Introduced in July 2004, *CattleMaster GOLD* is the first killed BVD vaccine licensed in the U.S. with label claims for use as an aid in preventing persistently infected calves caused by fetal infection with BVDV Types 1 and 2. Additionally, *CattleMaster GOLD* has a label claim for aiding in the prevention of IBR abortion. Presented here is a summary of the *CattleMaster GOLD* efficacy studies demonstrating protection against the birth of PI calves caused by fetal infection with BVDV Types 1 and 2.

Study Overview: BVDV Fetal Protection

Test Vaccine

Immune responses to the BVDV Type 1 and Type 2 challenge viruses were stimulated by vaccine formulated to contain only the minimum immunizing dose (MID) of either the BVDV Type 1 or BVDV Type 2 vaccine antigens. When pregnant cows were challenged with BVDV Type 1, they were vaccinated prebreeding with *CattleMaster GOLD FP® 5* specially formulated with commercial levels of IBR (BHV-1), PI_3 virus, and BRSV, but with only MID levels of BVDV Type 1 and Type 2 virus. When pregnant heifers were challenged with BVDV Type 2, they were vaccinated prebreeding with *CattleMaster GOLD* containing only MID levels of BVDV Type 2 virus but commercial levels of BVDV Type 1 virus. MID levels are established prior to licensing of a vaccine and reflect an antigen level that would likely be present at product expiration. By using MID levels for challenged antigens, investigators put the challenge antigen at its maximum potential disadvantage. When a vaccine withstands challenge under these trying circumstances, it will likely be at least as effective when antigen level is at a normal release level. Commercial vaccine lots have substantially higher release titers to ensure potency throughout the product shelf life.

Study Design—BVDV Type 1 Challenge

In the first BVD fetal protection study, BVDV-seronegative cows were vaccinated with either *CattleMaster GOLD FP 5* (n = 19) or a placebo (n = 20) on Day 0 and Day 21. Following estrus synchronization, all test cows were bred by artificial insemination with certified BVDV-negative semen at approximately two weeks after the Day 21 vaccination. At approximately 60 days of gestation, pregnancy status was confirmed by means of rectal palpation. All pregnant cattle, 10 in each group, were challenged intranasally on Day 117 by instillation of a 4 mL dose of virulent heterologous noncytopathic BVDV Type 1 (Strain 816317, New York field isolate). On Study Day 145, amniocentesis was performed, and, at approximately 8.5 months of gestation, caesarean sections. BVDV isolation was performed on cow blood, amniotic fluid, and fetal tissues, and BVDV immunohistochemistry on fetal tissues.

Study Design—BVDV Type 2 Challenge

BVDV-seronegative crossbred beef heifers were vaccinated with either *CattleMaster GOLD* (n = 20) or a placebo (n = 19) on Day 0 and Day 21 (approximately 5 and 2 weeks prior to breeding). Following estrus synchronization, all test animals were bred by artificial insemination at approximately two weeks after the Day-21 vaccination with semen from a donor negative for BVDV and IBR virus. Pregnancy status was confirmed by ultrasound at approximately 50 to 52 days of gestation and again at 79 to 81 days of gestation. All pregnant cattle, 11 in the *CattleMaster GOLD* group and 8 in the placebo group, were challenged intranasally on Day 119 (80 to 82 days of gestation) by instillation of a 4 mL dose of virulent heterologous noncytopathic BVDV Type 2 (Strain 94B-5359a, Wyoming field isolate). At 155 to 157 days of gestation (Study Day 194), aseptic fetal harvests were performed following fetal euthanasia. BVDV isolation was conducted on cow blood and fetal tissues (brain, liver, lung, and spleen).

Results

BVDV Type 1—Serology

As Figure 1 shows, *CattleMaster GOLD* vaccinates had significantly ($P \leq 0.05$) higher BVDV Type 1 serum virus neutralizing (SVN) geometric mean titers (GMTs) at all sampling days after vaccination. All placebo controls remained seronegative until challenge day (Day 117). On Day 145 (28 days after challenge), the BVDV Type 1 SVN GMTs in all treatments increased over prechallenge titers, indicating a serologic response to challenge.

BVDV Type 1—Clinical and Laboratory Observations

Table 1 summarizes the postchallenge virus isolation and immunohistochemistry results. All vaccinates were BVDV isolation negative on all 8 postchallenge blood collection days. BVDV was isolated from blood after challenge at a significantly ($P \leq 0.05$) lower frequency from vaccinates (0%) than controls (90%). None of the fetal tissues from vaccinates (0%) were BVDV virus isolation positive whereas all (100%) of the fetal tissues from placebo cows were positive, a significant ($P \leq 0.05$) difference.

Amniotic fluid samples from 0% of vaccinates were BVDV isolation positive. In comparison, BVDV was isolated from the amniotic fluid of all control cows (100%), also a significant ($P \leq 0.05$) difference. All fetal tissues harvested from vaccinates (100%) were BVDV immunohistochemistry negative. Thus, BVDV was detected at a significantly ($P \leq 0.05$) lower frequency from fetuses of vaccinates (0%) than controls (100%).

BVDV Type 2—Serology

As Figure 2 shows, *CattleMaster GOLD* vaccinates had significantly ($P \leq 0.05$) higher BVDV Type 2 SVN GMTs on Day 21, 35, and 119 after vaccination. All placebo controls remained seronegative until challenge day (Day 119). On Day 194 (75 days after challenge), the BVDV Type 2 SVN

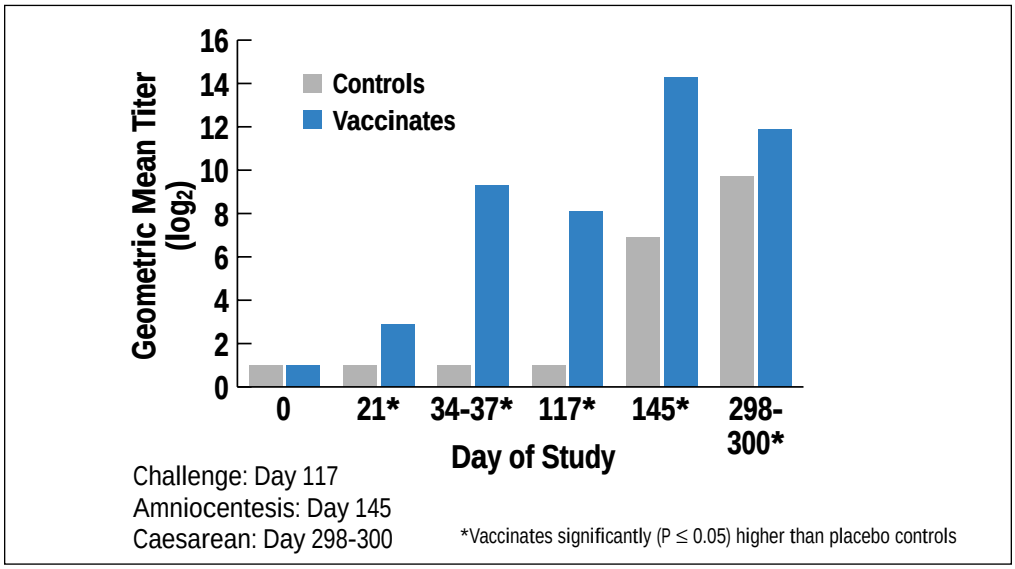

Figure 1 – Postvaccination BVDV Type 1 serum virus neutralization titers

Table 1–Summary of postchallenge cow and fetal BVDV isolation results (BVDV Type 1 challenge)

	Postchallenge virus isolation method and incidence			
Vaccine	**Viremia Cows***	**Amniotic Fluid Virus Isolation**	**Fetal Tissue Virus Isolation†**	**Fetal Tissue Immunohistochemistry†**
Controls	90%	100%	100%	100%
CattleMaster GOLD FP 5	0%[a]	0%[a]	0%[a]	0%[a]

*Virus isolation was attempted from buffy coat cell preparations from samples collected at 9 intervals from day of challenge (Day 117) to Day 145. A cow was considered viremic if any blood sample was BVDV positive.
†Fetal tissues were collected following abortion or Caesarean section. A fetus was considered BVDV positive if any tissues were positive.
[a]Significant ($P \leq 0.05$) difference versus placebo group.

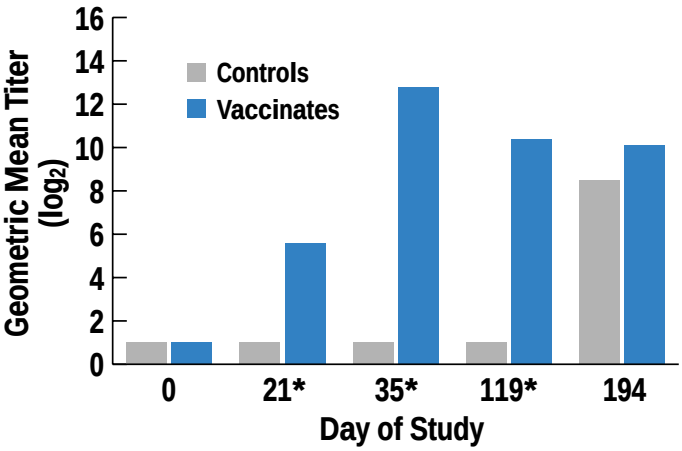

Figure 2 – Postvaccination BVDV Type 2 serum virus neutralization titers

GMTs in all treatments increased over prechallenge titers, indicating a serologic response to challenge.

BVDV Type 2—Clinical and Laboratory Observations

Following challenge, there were no significant differences in febrile response between vaccinates and controls, an outcome consistent with that observed in previous studies using BVDV Type 2 strain 94B-5359a challenge virus. Also consistent with challenge studies using this challenge strain, no animals developed clinical signs of disease. This observation underscores the subtlety of fetal infection, which can occur without any outward signs of clinical disease.

Table 2 summarizes the postchallenge virus isolation results in dams and fetuses. Vaccinated animals experienced a significant (P ≤ 0.05) reduction in both the frequency and duration of viremia following challenge. During the 8-day observation period, the least squares (LS) mean percent of days that controls were viremic was

Least Squares Mean Percent Days: A Brief Explanation

The term "Least Squares (LS) Mean Percent Days" is used by Pfizer scientists to help characterize the extent that a particular disease parameter was observed in groups of test animals following challenge. LS mean percent days factors in the time intervals that animals are affected as well as the percentage of animals affected. In brief, "LS mean percent days" is the average percent of days animals in a treatment group were afflicted with a given condition.

Table 2–Summary of postchallenge dam and fetal BVDV isolation results (BVDV Type 2 challenge)

	Postchallenge viremia—dams and fetuses		
Vaccine	Viremia Dams*	Least Squares Means % Days with Viremia Dams	Fetal Tissue Virus Isolation†
Controls	100%	44.4	88.0%
CattleMaster GOLD	27%[a]	1.2[a]	0%[a]

*Virus isolation was attempted from blood samples collected on Day 119 and Day 122 to 129. The presence of BVDV was determined after two passages in cell culture using an indirect immunofluorescence assay. An animal was considered viremic if any blood sample was BVDV positive.

†Fetal tissues were collected following abortion or fetal euthanasia. A fetus was considered BVDV positive if any tissue was positive.

[a]Significant (P ≤ 0.05) difference versus placebo group.

44.4%. In comparison, the vaccinate group was viremic for only 1.2% days, a significant ($P \leq 0.05$) difference and a reduction of 97.3%. Fetal tissues from all vaccinates (100%) were BVDV virus isolation negative whereas 88.0% of fetal tissues from control animals were positive, which was also significantly ($P \leq 0.05$) different.

Conclusion and Discussion

Prebreeding vaccination of BVDV-seronegative cows with *CattleMaster GOLD* protected 100% of their calves from becoming persistently infected following virulent challenge with BVDV Type 1 or BVDV Type 2. These results are particularly noteworthy because previous studies attempting to demonstrate efficacy of inactivated vaccines against fetal challenge produced either varying degrees of success or used a BVDV challenge that failed to consistently affect control animals.[8-13] Results obtained in the *CattleMaster GOLD* studies were the basis for obtaining the first BVDV Type 1 and Type 2 fetal protection claim for a killed BVDV vaccine licensed in the U. S.

The difficulty of achieving this level of fetal protection is also noteworthy. First, it is important to recall that BVDV has a strong tropism for tissues of the bovine reproductive tract.[5,13-16] Even minimal cross-placental virus transfer is sufficient to produce fetal infection.[15] In the *CattleMaster GOLD* studies, the difficulty of establishing protection was further enhanced by the challenge model. The intranasal challenges not only mimicked the natural route of infection, but also were at dosages much greater than would be expected from field exposure. In the face of these obstacles, 100% of the fetuses from cows vaccinated with *CattleMaster GOLD* were refractory to challenges that produced 100% fetal infection in controls exposed to BVDV Type 1 and 88% fetal infection in controls exposed to BVDV Type 2. Such an achievement with inactivated BVDV agents represents a significant advance in efforts directed at the control of BVDV herd infections and associated economic losses.

References

1. Data on file, Study 3131C-60-96-154, Pfizer Inc.

2. Data on file, Study 3131R-60-02-250, Pfizer Inc.

3. Bolin SR, McClurkin AW, Coria MF. Frequency of persistent bovine viral diarrhea virus infection in selected cattle herds. *AJVR* 1985;46(11):2385-2387.

4. Howard CJ, Brownlie J, Thomas LH. Prevalence of bovine virus diarrhea virus viraemia in cattle in the UK. *Vet Rec* 1986;119(25):628-629.

5. Wittum TE, Grotelueschen DM, Brock KV, et al. Persistent bovine viral diarrhea virus infection in US beef herds. *Preventive Veterinary Medicine* 2001;49:83-94.

6. Baker JC. Bovine viral diarrhea virus: A review. *JAVMA* 1987;190:1449-1458.

7. Cortese VS, Grooms DL, Ellis J, et al: Protection of pregnant cattle and their fetuses against infection with bovine viral diarrhea virus type 1 by use of a modified-live virus vaccine. *AJVR* 1998;59:1409-1413.

8. Bolin SR, Littledike ET, Ridpath JF. Serologic detection and practical consequences of antigenic diversity among bovine viral diarrhea viruses in a vaccinated herd. *AJVR* 1991;52:1033-1037.

9. Harkness JW, Roeder PL, Drew T, et al. The efficacy of an experimental inactivated BVD-MD vaccine. *Agric Pestivirus Infect Rumin* 1987;233-250.

10. Kaeberle ML, Maxwell D, Johnson E. Efficacy of inactivated bovine viral diarrhea virus vaccinates in a cow herd. *Anim Sci Leaflet R701* Ames, Iowa: Iowa State University Press, 1990;42-43.

11. Ellsworth MA, Kelling CL, Dickinson EO, et al. Fetal infection following bovine viral diarrhea virus challenge of vaccinated and nonvaccinated dams. In: *Proceedings, 74th Conference of Research Workers in Animal Disease,* 1994;34.

12. Meyling A, Rensholt T, Dalsgaard K, et al. Experimental exposure of vaccinated and nonvaccinated pregnant cattle to isolates of bovine viral diarrhea virus (BVDV). *Agric Pestivirus Infect Rumin* 1987;225-231.

13. Brownlie J, Clarke MC, Hooper LB, et al. Protection of the bovine fetus from bovine viral diarrhoea virus by means of a new inactivated vaccine. *Vet Rec* 1995;137:58-62.

14. Orban S, Liess B, Hafez SM, et al. Studies on transplacental transmissibility of a bovine virus diarrhoea (BVD) vaccine virus. I. Inoculation of pregnant cows 15 to 90 days before parturition (190th to 265th day of gestation). *JVMB* 1983;30:619-634.

15. Dufell SJ, Harkness JW. Bovine virus diarrhea-mucosal disease infection in cattle. *Vet Rec* 1985;117:240-245.

16. Roeder PL, Harkness JW. BVD virus infection: Prospects for control. *Vet Rec* 1986;118:143-147.

17. Ficken M. Jeevaraerathnam S, Wan Welch SK, et al. BVDV fetal infections with selected isolates. In: *Proceedings of the International Symposium on Bovine Viral Diarrhea Virus,* a Fifty-Year Review. Ithaca, NY, 1996;110-112.

CMR04023

Pfizer Animal Health