Pfizer Animal Health
Technical Bulletin

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CattleMaster® GOLD™: Severe respiratory challenge confirms efficacy of killed BVDV Type 2 fraction

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Key Points

- An efficacy study was conducted to evaluate the ability of the inactivated BVDV Type 2 fraction of *CattleMaster® GOLD™ FP® 5* to protect BVDV-seronegative calves against severe respiratory disease caused by intranasal challenge with virulent BVDV Type 2 (Strain 24515).[1,2]
- Following challenge, clinical signs were observed in 100% of the controls. The least squares (LS) mean percent days with clinical disease was reduced by more than 99% by vaccination with *CattleMaster GOLD*.
- *CattleMaster GOLD* prevented viremia in 100% of vaccinates, whereas 100% of controls developed BVD viremia following challenge.
- *CattleMaster GOLD* vaccinates were protected against the immunosuppressive effects of the BVDV challenge. Following challenge, the LS mean percent days with leukopenia for controls was 59.6, compared with only 0.70 for vaccinates, a reduction of 98.8%.
- No vaccinates died following challenge, whereas 83% of the controls either died or had to be humanely euthanized as a result of severe BVD disease.

The importance of bovine viral diarrhea virus (BVDV) Type 2, the second genotype of BVDV, has only recently gained recognition, although the virus has been present in the cattle population for many years. BVDV Type 2 can produce the same acute respiratory-enteric-immunosuppressive syndrome as BVDV Type 1,[3,4] but with varying degrees of severity.[5,6] Some investigators have found BVDV Type 2 challenge isolates to be more virulent than BVDV Type 1 in producing acute and peracute disease;[7] others that the BVDV Type 2 strains causing severe acute disease are in the minority and that most BVDV Type 2 strains are no more virulent than BVDV Type 1 strains.[8] BVDV Type 2 also produces BVD hemorrhagic syndrome, a possibly underreported and often severe disease identified in the U.S. within the past fifteen years.[4,5,9,10]

Introduced in July 2004, *CattleMaster GOLD* is the first killed BVD vaccine licensed in the U.S. that has label claims for use as an aid in the prevention of BVDV persistent infection (Types 1 and 2) and respiratory

diseases caused by BVDV Types 1 and 2. Presented in this bulletin is a synopsis of the *CattleMaster GOLD* study demonstrating protection against respiratory disease caused by virulent BVDV Type 2 challenge.

Study Overview: BVDV Type 2 Respiratory Protection

Test Vaccine

The vaccine used in the BVDV Type 2 respiratory protection study was formulated with a minimum immunizing dose (MID) level of killed BVDV Type 2 and commercial levels of BVDV Type 1. MID levels are established prior to licensing of a vaccine and reflect an antigen concentration 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 vaccine withstands challenge under these trying circumstances, it will likely be at least as effective when antigen level is at a normal release dose. Commercial vaccine lots have substantially higher release titers to ensure potency throughout product shelf life.

Study Design—BVDV Type 2 Respiratory Challenge

BVDV-seronegative and virus-free crossbred calves 2.5- to 5.5-months-old were vaccinated with a 2 mL dose (n = 12) of *CattleMaster GOLD* or a 2 mL dose of placebo (n = 12) on Day 0 and Day 21. On Day 42, all calves were challenged intranasally with a 5 mL dose (approximately 2.5 mL per nostril) containing a mean titer of 5.25 $\log_{10}$ $FAID_{50}$* virulent noncytopathic BVDV Type 2 (Strain 24515).

Serology

Blood samples were collected from all study animals on Days 0, 21, 35, 42, and 56 for determination of BVDV Type 1 and BVDV Type 2 serum virus neutralizing (SVN) antibodies. On Days 0, 21, and 42, samples were obtained prior to vaccination or challenge.

*$FAID_{50}$ = fluorescent antibody infectious dose; assay method for quantifying titers of BVDV Types 1 and 2; an alternative assay comparable to tissue culture infectious dose ($TCID_{50}$) units.

Rectal Temperature

Rectal temperatures (°F) were obtained and recorded on Days 40 through 56, inclusive.

Clinical Scoring

Clinical disease scores of 0, 1, 2, or 3, based on clinical signs attributable to BVDV Type 2 infection, were made for each animal on Days 40 through 56, with challenge day scores (Day 42) recorded prior to administration of challenge virus. The clinical scoring model is described in Table 1.

Table 1–Clinical observation scoring criteria (BVDV Type 2 respiratory challenge)

0 = NORMAL ANIMAL
- No clinical signs

1 = NONSPECIFIC CLINICAL SIGNS
- Clinical signs as a whole are not specific for acute BVD infection. Clinical signs may include nasal discharge, abnormal respiration, and lethargy.

2 = ACUTE BVD CLINICAL DISEASE
- Clinical signs as a whole are moderate in degree and specific for acute BVD infection. Clinical signs may include nasal discharge, abnormal respiration, lethargy, gauntness, ocular discharge, hypersalivation, diarrhea, dehydration, lameness and/or reluctance to move.

3 = SEVERE CLINICAL DISEASE
- Clinical signs as a whole are severe in degree and specific for acute BVD infection. Clinical signs may include nasal discharge, abnormal respiration, lethargy, gauntness, ocular discharge, hypersalivation, diarrhea, excessive bruising, dehydration, recumbency, lameness, and/or reluctance to move.

Virus Isolation

Blood samples were collected from each animal on Day 42 and Days 45 through 52, inclusive. The Day 42 samples were collected prior to challenge. BVDV isolation was performed on buffy coat cells separated from whole blood.

White Blood Cell Counts—Leukopenia

Blood samples were collected from all study animals on Days 40 through 42 and 45 through 52, inclusive, for determination of total white blood cell (WBC) counts. The Day 42 sample was obtained prior to challenge. Leukopenia was defined as a ≥ 40% reduction in the total WBC count compared to baseline counts (Days 40-42).

Data Analysis

A mixed linear model with repeated measures was used to analyze BVDV SVN titers, virus isolation, percent of days for each animal with disease, rectal temperatures, WBC counts, and leukopenia. Fisher's exact test was used to analyze the incidence of animals with disease. With each analysis, pairwise comparisons were made between the control group and the treatment group. The 5% level of significance ($P \leq 0.05$) was used to assess statistical differences for all tests.

Results

Serology

As Figure 1 shows, *CattleMaster GOLD* vaccinates had significantly ($P \leq 0.05$) higher BVDV Type 2 serum virus neutralizing (SVN) geometric mean titers (GMTs) at all sampling days after vaccination. All placebo controls remained seronegative until challenge day (Day 42). Calves administered *CattleMaster GOLD* had a SVN GMT to BVDV Type 2 of 11.9 (log_2) at challenge on Day 42.

Least Squares Mean Percent Days: A Brief Explanation

The term "Least Squares (LS) Mean Percent Days" is used in this bulletin in characterizing rectal temperatures, clinical signs, viremia, and leukopenia that were observed in groups of test calves following challenge with BVDV Type 2. 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.

Rectal Temperature

One hundred percent of controls and 25.0% of vaccinates were febrile following challenge. During the 14-day postchallenge period, the maximum LS mean rectal temperature for vaccinates occurred on Day 45 and for controls on Day 51 (Figure 2). In the control group, the maximum LS mean rectal temperature was 106.5°F; in animals administered *CattleMaster GOLD*, 103.5°F.

In the preceding analysis, fever was defined by more stringent standards for vaccinates than controls, in accordance with regulatory directives. Controls with temperatures ≥ 104.5°F were considered febrile, whereas vaccinates were considered febrile at ≥

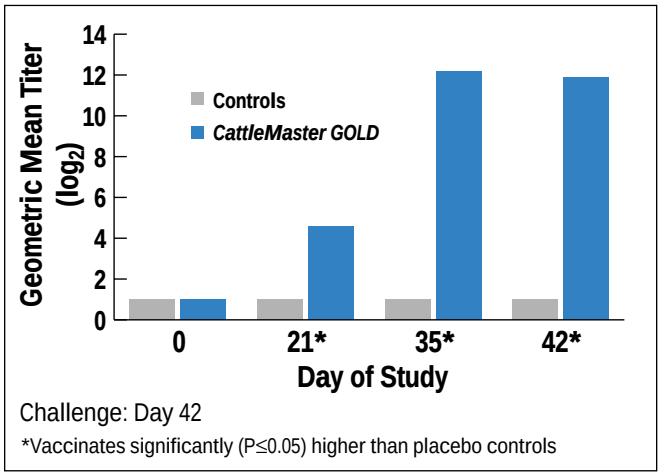

Figure 1 – Postvaccination BVD Type 2 serum virus neutralization titers

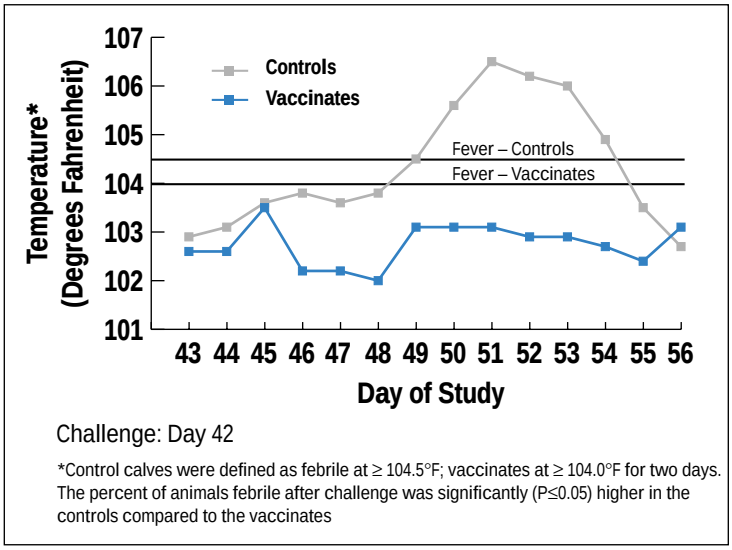

Figure 2 – Least squares mean rectal temperatures following challenge

Table 2–Least squares mean percent days with fever

	LS Mean % Days with Fever	
Group	**Fever Defined as ≥ 104.0°F**	**Fever Defined as ≥ 104.5°F**
Controls	42.7	32.4
CattleMaster GOLD	3.1[a]	0.5[a]

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

104.0°F for any two post-challenge days. Table 2 presents the results of analyses conducted with the same rectal temperature data when the identical fever criteria are applied to both controls and vaccinates, first with fever defined as ≥104.0°F and then as ≥ 104.5°F.

When fever was defined as ≥104.0°F, the LS means percent of days that controls and vaccinates were febrile was 42.7 and 3.1, respectively. The difference was significant (P ≤ 0.05) and represents a reduction of 92.7%. In the second analysis where fever was defined as ≥ 104.5°F, the LS mean percent days the control group was febrile was 32.4, and the vaccinate group 0.5, a significant (P ≤ 0.05) difference. Vaccination with *CattleMaster GOLD* reduced the percent days febrile by 98.5%.

Clinical Signs

Prior to and following challenge (Days 40 through 56), animals were scored daily for the presence of clinical signs attributable to BVD disease. Categorically, if an animal scored ≥ 2 on a 0 to 3 scale, it was regarded as demonstrating BVD. The frequency of animals with disease and the frequency of observations of disease are summarized in Table 3. Following challenge, clinical disease was observed in 100% of the controls and in only 8.3% of the calves administered *CattleMaster GOLD*, a significant (P ≤ 0.05) difference that represents a 92% reduction in clinical signs. The percent days with disease for controls was 28.1 compared with only 0.1 for vaccinates, a significant (P ≤ 0.05) difference and a reduction of > 99% with vaccination.

Viremia

Following challenge with BVDV Type 2, virus was isolated from 100% of the controls, and the LS mean percent days with viremia was 79. Strikingly, no virus could be isolated from any of the vaccinates at any sampling time, indicating there was no evidence of BVDV actively circulating in the animals (Table 4). Severity of the BVDV Type 2 is demonstrated by the LS means percent of days viremic for the control calves. The percent days viremic for the control group was 79, indicating great frequency and duration of viremia.

Leukopenia

Leukopenia is an abnormal decrease in the number of white blood cells, a condition that is often regarded as a measure of immunosuppression. All controls were leukopenic following challenge (Figure 3), and the LS mean percent days with leukopenia

Table 3–Clinical signs of BVD following challenge with BVDV Type 2 (strain 24515)

	Clinical Disease Scores ≥ 2	
Group	**Frequency of Animals Affected**	**LS Mean % Days with Disease**
Controls	100%	28.1
CattleMaster GOLD	8.3%[a]	0.1[a]

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

Table 4–BVD virus isolation following challenge

	BVD Virus Isolation	
Group	**Frequency of Viremic Animals**	**LS Mean % Days with Viremia**
Controls	100%	79.0
CattleMaster GOLD	0.0%[a]	0.0[a]

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

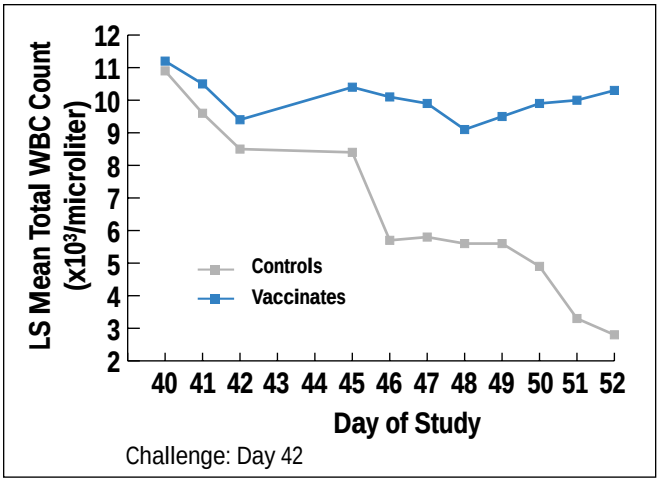

Figure 3 – Least squares mean total white blood cell count following challenge

was 59.9. In comparison, only 16.7% of vaccinates were leukopenic, and the LS mean percent days with leukopenia was 0.70 (Table 5), both indicating a significant ($P \leq 0.05$) difference compared with the control group calves. Vaccination with *CattleMaster GOLD* reduced the percent days with leukopenia by 98.8% compared to controls.

Mortality

Following challenge, 100% of the control calves showed clinical signs of BVD disease and 83% either died or were humanely euthanized as a result of severe BVD. Of the 10 dead animals, 7 died during the challenge phase (Day 42 through 56) and 3 were euthanized at the termination of the study (Day 56). Necropsy of each calf dying during the challenge phase revealed gross and histopathologic findings consistent with a severe, acute hemorrhagic BVD infection (data not shown). In comparison, no calves in the vaccinate group died or had to be euthanized (Table 6).

Conclusion and Discussion

CattleMaster GOLD vaccinates demonstrated a high level of protection against a very severe BVDV Type 2 challenge. The results are particularly noteworthy in view of the challenge model, which mimicked the natural intranasal route of infection and likely far exceeded what the animals would encounter in nature. Moreover, the challenge virus (Strain 24515) is widely recognized for its virulence. In response to the same challenge, 100% of the control cattle became viremic, had rectal temperatures greater than 104.5°F (≥ 40.3°C), developed clinical signs of BVD, and demonstrated profound leukopenia. Altogether, 83% of the control calves either died or were humanely euthanized as a result of the BVD disease.

The rectal temperatures, clinical disease scores, BVDV isolation, hematology, and mortality data demonstrate that *CattleMaster GOLD* vaccinates were solidly protected following severe challenge. Vaccination with *CattleMaster GOLD* reduced the percent days febrile by 92% to 98%, the percent days with clinical disease by 99%, the percent days viremic by 100%, and the percent days leukopenic by 98.8%

Table 5–Leukopenia following challenge

	Leukopenia*	
Group	**Frequency of Leukopenic Animals**	**LS Mean % Days with Leukopenia**
Controls	100%	59.6
CattleMaster GOLD	16.7%[a]	0.7[a]

*Leukopenia = ≥ 40% reduction in WBC on any day after challenge as compared to the prechallenge base line (Days 40-42).
[a]Significant ($P \leq 0.05$) difference versus the control group.

Table 6–Mortality following challenge

	Frequency of Dead Calves	
Group	**Day 42-Day 56**	**Euthanized Day 56**
Controls	58%	25%
CattleMaster GOLD	0%	0%

($P \leq 0.05$). Thus, based on the results of this study, a minimum immunizing dose of adjuvanted BVDV Type 2 antigen in *CattleMaster GOLD* was efficacious against a BVDV Type 2 challenge that caused severe disease in nonvaccinated control calves.

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