## **Research Summaries**

## Protocol for Testing Bovine Persistently Infected with BVD

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Bovine viral diarrhea virus can be consistently isolated from serum, buffy coat, nasal, and vaginal swabs from persistently infected animals (PIA). Using this information and the assumption that persistently infected calves, a protocol was developed to screen herds for persistently infected animals. Beef herds are screened by collecting blood and/or swabs from all calves, open females, and bulls. Dams of positive calves are then tested, avoiding the necessity of testing all of the cows in the herd. All positive animals are removed prior to the breeding season. In dairy herds, calving usually occurs throughout the year. All animals with herd contact should be tested and positive animals removed. All newborn calves should be screened for nine months. This protocol was developed while testing six herds, including both beef and dairy, involving over 1,100 animals.

#### Observations:

- Identification of all animals is essential.
- Prompt removal of PIA is required.
- PIA can be identified from blood and/or swabs.
- Proper collection and transportation of swabs to the laboratory is important. With proper instructions, a herdsman can collect satisfactory samples at birth. This avoids postnatal infection with BVDV.
- Most PIA were identified as calves; however, five 12–13 mo., one 16 mo., and one 26 mo. old heifer were identified. One PIA lived more than three years.
- Mucosal disease may appear clinically as wasting disease of a long duration.
- PIA can produce antibodies to BVDV; therefore, the presence of BVD antibodies cannot be used to eliminate animals as being persistently infected.

## **Bovine Spongiform Encephalopathy**

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Bovine Spongiform Encephalopathy (BSE) has been included in the group of diseases referred to as the slow viral encephalopathies because it has numerous similarities: an extended incubation with a short invariably fatal clinical course,<sup>1</sup> histopathology of neuronal degeneration and vacuolation,<sup>2</sup> the presence of scrapie associated fibrils<sup>2</sup> and experimental transmission to mice by intracerebral inoculation.<sup>3</sup>

Analysis of blood samples from 20 cases of BSE revealed no significant departure from normal values. Cerebrospinal fluid (CSF) analysis indicated an intact

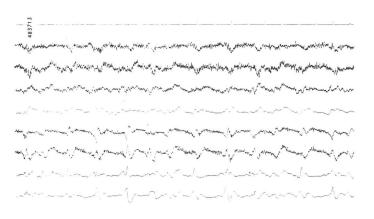
The Research Summaries were presented at the AABP Annual Convention in Kansas City on Wednesday, November 15, 1989. blood brain barrier and no intrathecal inflammatory response. This lack of host immune response is characteristic of the slow viral encephalopathy group of diseases.

Electroencephalographic (EEG) studies in ten normal cows, aged 3–6 years, revealed traces with a reasonably organized background activity of predominantly alpha frequency with wave fluctuation in the range of  $50-100 \,\mu$ V.

In the EEG traces recorded during the early clinical stages of BSE there are periods of normal activity interspersed with periods of increased amplitude background activity, up to  $200\mu V$  most marked posteriorly. Clear, sharply contoured waveforms are prominent in the posterior regions. In addition, there are brief bursts of complex activity.

Later there is persistent high amplitude activity in the

FIGURE 1. This EEG trace of a human patient in the terminal stages of Creutzfeldt-Jakob disease (CJD) shows disorganized background activity with periodic complexes occurring at a rate of approximately 1 per second.



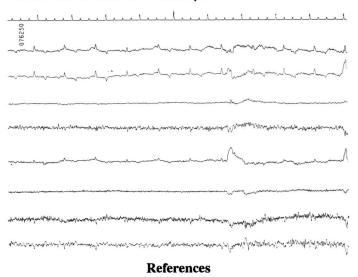
posterior leads and the appearance of disorganized activity in anterior leads.

Similar EEG trace changes to those observed in Figure 2 were recorded in the same cow six weeks later.

#### Summary

The lack of a host immune response presents a considerable obstacle to the veterinarian in the specific antemortem diagnosis of BSE. Electroencephalo-studies in this series provide the basis for further work which may lead to a definitive antemortem confirmatory test.

FIGURE 2. Normal activity is attenuated with little difference between activity in the anterior and posterior leads. Periodic complexes are clearly defined and occur singly or in clusters approximately 1 per second. Complex activity was accentuated following the intravenous administration of diazepam.



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# Results of a New Single Injection Antibiotic for the Treatment of Bovine Respiratory Disease.

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Tilmicosin is a new long acting macrolide antibiotic developed to treat bovine respiratory disease (BRD). It has broad spectrum activity against several common bacterial pathogens, including the Pasturella sp. that are implicated in the Shipping Fever complex.

Several studies were conducted in western Canada in 1986–1989, to evaluate the effectiveness of this product against BRD in recently weaned auction market derived feedlot calves. These calves originated from cow/calf herds throughout western Canada, weighed between 225 kg and 350 kg body weight and ranged from eight to eleven months in age. Animals selected for the trials were suffering clinically from BRD, had no previous feedlot treatment history and had a rectal temperature greater than 105 degrees Fahrenheit (40.5 C).

Initially, dose titration studies were undertaken to establish appropriate dosage levels for tilmicosin. Cattle fitting the case definition were randomly blocked into groups of four head. One animal became a negative control while the other three calves received tilmicosin at 5, 10 and 20 mg/kg body weight. Temperatures returned to normal within 24 hours following treatment in all groups

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treated with tilmicosin. The mortality data of three trials is summarized in Table 1. Ten of 27 calves (37%) in the negative control group died, one of 27 calves (3.7%) in the 5 and 10 mg/kg tilmicosin treated groups died, while no deaths occurred in the 20 mg/kg treated group.

Tilmicosin was also evaluated for the treatment of BRD against other currently available antimicrobials. Two studies were completed, one involving sodium ceftiofur and the other involving trimethoprim-sulfa. The trial results are summarized in Tables 2 and 3. In both trials, tilmicosin

TABLE 1. Summary of Tilmicosin dose titration data (3 trials).

Tilmicosin level	Died/Treated	% Died
Placebo 5 mg/kg 10 mg/kg	10/27 1/27 1/27	37.0 3.7 3.7
20 mg/kg	0/27	0.0

TABLE 2. The comparative effectiveness of Tilmicosin and Sodium Ceftiofur for the treatment of BRD.

	Tilmicosin	Sodium ceftiofur
No. of cases	159	158
Treatment days (initial)	159	474
No. of first repulls	98	107
Treatment days (1st repull)	98	321
No. of second repulls	16	34
Treatment days (2nd repull	) 16	102
Total treatment days	272	897
Average treatment days	1.71	5.68
Mortality (all cases)	1	4
Mortality (fibrinous pneumonia) 1		3

treated cattle had fewer relapses, were treated fewer days on average and required fewer total treatment days. There were fewer deaths due to fibrinous pneumonia in the tilmicosin treated cattle.

Tilmicosin was compared with Long Acting Oxytetracycline and a negative control group of animals against BRD. The results are summarized in Table 4. Animals fitting the case definition were randomly blocked onto the trial. Treated cattle were injected once with antibiotic, returned to their home pen and subsequently monitored for 28 days. Thirty percent (30%) of the untreated negative controls died while only 12% of the animals died in each of the treated groups.

In summary, tilmicosin is safe and effective in treating BRD in feedlot cattle. Its long acting effect gives it several advantages in terms of reducing overall treatment days, relapse rate and average treatment days over shorter acting antibiotics used for the same purpose.

TABLE 3. The comparative effectiveness of Tilmicosin and Trimethoprim-sulfa for the treatment of BRD.

	Tilmicosin	Trimethoprim- sulfa
No. of cases	167	162
Treatment days (initial)	167	486
No. of first repulls	16	45
Treatment days (1st repull)	16	135
No. of second repulls	1	13
Treatment days (2nd repull)	1	38
Total treatment days	184	659
Average treatment days	1.10	4.07
Mortality (all cases)	3	4
Mortality (fibrinous pneumoni	ia) 0	2

TABLE 4. Comparison of Tilmicosin and long acting Oxytetracycline for the treatment of BRD.

Treatment	Died/Treated	% Died
Negative Control	3/10	30
Tilmicosin 10 mg/kg	3/25	12
LA Oxytetracycline 20 mg/k	g 3/25	12

# Isolation of *Mycobacterium paratuberculosis* from Washed Bovine Ova After In Vitro Exposure.

Ronald F. Rohde, DVM, MS

To test the efficacy of a 10-step wash procedure for removing Mycobacterium paratuberculosis from bovine ova, washed zona pellucida intact (ZPI) bovine ova were incubated in Dulbecco's phosphate buffered saline (DPBS) supplemented with 2% fetal bovine serum (FBS) containing concentrations of  $10^4$ ,  $10^3$ ,  $10^2$ ,  $10^1$  and  $10^0$  colony forming units of Mycobacterium paratuberculosis per ml for 12 hours at room temperature. Ten ZPI ova were removed from each concentration and washed by passing through 10 changes of DPBS supplemented with 15% FBS. Each wash step was a 1:100 dilution of the previous step with the tenth step representing a 1:10<sup>20</sup> dilution of the initial concentration. The media from each wash step was inoculated onto 3 slants of Herrold's egg yolk medium supplemented with sodium pyruvate and mycobactin J and one slant without mycobactin J. The ova were included with the tenth wash step.

One colony of *Mycobacterium paratuberculosis* was isolated from 1 of 10 tenth wash steps at the  $10^4$  concentration

and 5 of 10 tenth wash steps at  $10^3$ . Organisms were not recovered from wash steps 3 through 9 for all concentrations studies except for once at the sixth step from the  $10^3$  concentration. These observations suggest that the organisms recovered from the tenth wash steps were associated with the ova.

This study indicates that if embryos were exposed to *Mycobacterium paratuberculosis* in the uterus of an infected donor cow, the 10 step embryo wash procedure might not be efficacious in removing the organism from recovered embryos. These embryos could then potentially serve as a vehicle of transmission of the organism to the recipient animal.

In a related study, acid-fast organisms with cultural characteristics similar to *Mycobacterium paratuberculosis* were isolated from uterine flush fluids of three of four cows with clinical paratuberculosis following standard uterine flush procedures.

## **Caudal Epidural Analgesia After Xylazine Injection in Cows**

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Xylazine (0.05 mg/kg of B.W. diluted to a 5 ml volume using 0.9% NaCl) or 5 ml of NaCl 0.9% solution was injected epidurally into the first caudal intervertebral space  $(C_1-C_2)$  in 8 cows. The cows were observed for responses to deep needle prick of the caudal dermatomes (S<sub>3</sub> to coccyx), sedation, and ataxia. Heart rate, respiratory rate, body temperature, rate of ruminal contractions, arterial blood pressure, arterial pH, blood gases (PaO<sub>2</sub>, PaCO<sub>2</sub>), base excess, total solids concentration, and hematocrit were determined before and after injections. Epidural xylazine induced sedation and long-lasting selective (S<sub>3</sub> to coccyx) analgesia for at least 2 hours. Mild ataxia of hind limbs was observed in 6 cows, but all cows remained standing. Heart rate, respiratory rate, rate of ruminal contractions, arterial blood pressure, PaO<sub>2</sub>, packed cell volume, and total solids concentration were significantly (P < 0.05) decreased, and PaCO<sub>2</sub>, base excess, and bicarbonate were significantly increased after xylazine injection. Epidural NaCl 0.9% solution did not alter sensory perception to needle prick and did not affect any of the physiologic parameters determined. Although epidural xylazine administration induced good analgesia and sedation in healthy cows, it should be avoided as an epidural analgesic in cattle with heart disease, lung disease, and/or gastrointestinal disease due to its potent cardiopulmonary and ruminal depressant effects.

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# Safety and Efficacy of a Bovine Sustained Release Selenium Device

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Selenium deficiency is a commonly recognized problem in cattle and is associated with a variety of clinical conditions. Currently available methods of supplementing deficient animals in feed or by injection are not practical in all management situations. A sustained release intraruminal bolus that releases 3 mg of selenium as sodium selenite per day for 120 days to cattle has been developed. The osmotically driven pump within the bolus reaches study state in 3–4 weeks, discharges a consistent 3 mg/day plateau for about 100 days and then rapidly shuts down in a few days. The bolus is weighted on one end to insure retention within the reticulum or rumen.

The bolus is delivered by balling gun and is safe and efficacious in cattle. Significant elevations in whole blood seleniums occur when the bolus is given to selenium deficient cows and calves and deficient animals become blood selenium normal within 30–40 days. Whole blood selenium levels continue to rise throughout the lifespan of the bolus and remain elevated months past its expiration. Blood selenium levels for calves peak higher than those for cows. The bolus has been given to calves as small as 200 lbs. without problems. Multiple boluses given to individual animals created no obstructions or medical problems. Intentionally damaged boluses allowing escape of all of the selenium matrix at once did not result in any toxicity. Retention rates in cattle receiving the bolus have been 96–100%.

The sustained release selenium bolus provides a consistent and safe means of supplementation in cattle. It will be particularly useful in management situations where current supplementation options are inconsistent or inconvenient such as in pastured or grazing animals and dry cows.

### Failure of a Sequential-Spring Strategic Deworming Program to Produce Parasitological or Weight Gain Changes in a Beef Cow/Calf Herd.

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#### Objectives

This study was designed to assess the results of a sequential-spring strategic deworming program applied to a beef cow/calf operation. Specifically, would this deworming program produce changes in fecal egg output patterns, serum pepsinogen levels, grass larval contamination, weight gains/losses of calves or cows, or reproductive performance of cows?

#### Results

One hundred thirty cow/calf pairs were randomly divided into three treatment groups. Each treatment group was further divided into two replicates. Each group was grazed on a separate pasture with equivalent stocking rate and estimated equal quality. In Treatment 1 cows and calves received an ivermectin (Ivomec®) treatment at turnout and again 5 weeks after turnout. In Treatment 2 calves only received a treatment with ivermectin at turnout and again at 5 weeks after turnout. In Treatment 3 no anthelmintic treatment was administered throughout the grazing season.

During the grazing season from April 28 to October 19, cows and calves were weighed every 5 weeks and fecal and serum samples were collected sequentially from randomly selected individuals. Herbage samples were collected at the same intervals and analyzed for number of *Ostertagia o*. larvae. Fecal samples were analyzed for the presence of trichostrongyle type eggs. Serum samples were analyzed for levels of serum pepsinogen.

Calves receiving treatment three (Control) had a tendency to have higher fecal egg counts throughout much of the grazing season but counts were not different at weaning. Control calves also had a higher serum pepsinogen level (P < .05) at weaning. Neither fecal egg counts for cows, serum pepsinogen levels for cows, pasture herbage larval levels, nor cow weight gain/loss profiles were different among groups. Non-pregnancy rates for cows in Treatment groups 1, 2 and 3 were 8.3%, 8.1% and 3.2% respectively. Total calf gains during the trial for Treatment

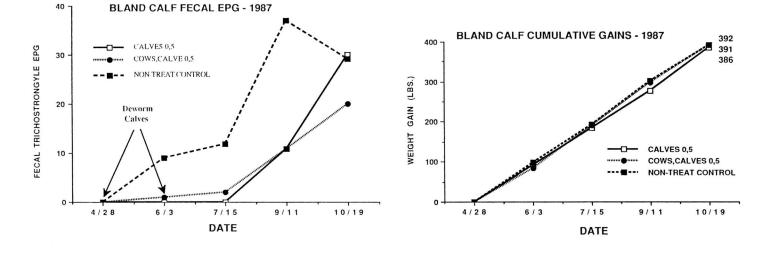
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groups 1, 2 and 3 were 177.9 kg, 175.5 kg and 178.1 kg respectively. These gain differences were non-significant

FIGURE 1. Output of trichostrongyle-type eggs in the feces of three groups of calves subjected to different deworming treatment regimes.

(P>0.86). Figures 1 and 2 are graphs depicting the fecal egg outputs and weight gains for calves in the study.

FIGURE 2. Cummulative weight gains for three groups of calves subjected to different deworming treatment regimes.



### Persistent Infections with Bovine Viral Diarrhea Virus in a Beef Herd

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The objectives of this study were to investigate the time course of natural bovine viral diarrhea virus (BVDV) infections and the effect on production in a range beef cattle herd, and to access methods for detection of persistentlyinfected (PI) cattle. Determinations were based on epidemiological data from virus isolation tests and serology of sequential blood serum specimens collected from calves at birth and at 6 week intervals to 10 months of age. Affected calves showed signs of infection between 2 and 3 months of age. Clinical signs included elevated body temperature, mild diarrhea and rapid respiratory rate. Lesions included reddening and erosions of the mucosa of the hard palate. Nine of the 143 calves were PI with BVDV and were the likely source of virus for the postnatal infection. Antibody titers of PI calves dropped precipitously by the time calves were 2 months old allowing detection of BVDV in serum

specimens of all PI calves at this time. Suspect PI calves were detectable serologically and were confirmed PI by virological examination of serum specimens 4 months after weaning when the calves were 9 months old. Growth rates were remarkably reduced in PI calves compared to the non-PI calves in the herd. Four of the five PI calves for which weights were available were the lightest and clearly the most unthrifty calves in the herd when they were 7 months old. Apparently, removal of unthrifty cattle from the primary herd is an effective means of limiting transmission of BVDV and exposure of susceptible cattle to BVDV. Laboratory testing cattle for persistent BVDV infections is necessary to ensure detection and removal of all PI cattle from a herd. The tests are costly, but are economically justifiable to reduce production costs due to BVDV infections.

### Active Immunization of Cows with a Salmonella Typhimurium Mutant Bacterin Toxoid and the Passive Transfer of Anti-Core-Antigen Antibodies in Colostrum

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Twenty normal Holstein and Guernsey cows in their last six weeks of gestation were injected with either a placebo or Salmonella typhimurium mutant bacterin-toxoid. The vaccine stimulated a significant (P < 0.05) increase in the mean anti-core-antigen serum antibody titers from 1:1,596 while there was no significant increase in serum antibody titers in placebo treated cows.

When mean anti-core-antigen antibody titer levels of colostrum from the cows were measured there was a significant (P < 0.05) difference between the placebo, 1:3,397,

## **For Your Library**

AGRICULTURAL POLICY REFORM Politics and Process in the EC and USA *H. Wayne Moyer, Timothy E. Josling* 

The 1980's were troubled times for agriculture in both the United States and the European Community (EC). This book identifies and analyzes the principal agricultural reform initatives during the 80's in the EC, the USA, and in the international trade arena. More specifically, Agricultural Policy Reform examines the role of the political process in explaining agricultural policy decisions. The book uses decision-making theories to explain why agricultural policy decisions depart from rationality and why reform is difficult. It discusses the growing pressure for the reform of the international system for agricultural trade and the link between trade reform and agricultural policy reform. It uses similar methods of analysis to provide the analytical framework for this comparative study of the problems and processes involved in reforming the agricultural sectors of the EC and USA.

Agricultural Policy Reform begins by developing an analytical framework for the assessment of agricultural policy decision making. Next, a detailed examination of the agricultural policy process in the USA and the EC follows with a discussion of the "reforms" of the 1980's. The decision-making processes are compared for the 1981 and 1985 US farm bills, the milk quotas decision of 1984, and the agricultural stabilizers agreement of 1988 to show the applicability of the analytical framework to specific policy and vaccinated 1:6,794, groups. Mean serum anti-coreantigen antibody titers of calves at 24 hours of age from the placebo treated and vaccinated were 1:832 and 1:1,448 respectively and significantly (P < 0.05) different.

It was concluded that cows vaccinated with Salmonella typhimurium mutant bacterin-toxoid during the last six weeks of gestation seroconverted in terms of anti-coreantigen antibodies and that they passively transferred significantly (P < 0.05) higher levels of these antibodies through colostrum to their calves.

situations. There is discussion on the pressures to reform the international system for agricultural trade. The final chapter is devoted to comparing and contrasting the EC and US experiences and looks for lessons for those charged with reforming farm policies in industrial countries.

Agricultural policy reform can only be achieved in a situation of budget crisis and will proceed only incrementally because of the necessity of reaching consensus through bargaining between diverse interests. *Agricultural Policy Reform* offers valuable new insights into the question of policy reform and will be essential reading not only for agricultural economists but also trade policy analysts and those interested in the theory and practice of the policy process. The nature of the subject will give the book an appeal to both scholars and professionals interested in agricultural policy.

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## Local Intravenous Anesthesia in Teat Surgery of Cattle

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Teat injuries usually are treated surgically under sedation and injection of a local anesthetic around the base of the teat (AMSTUTZ, 1980). A different method of anesthesia ia local intravenous anesthesia (SURBORG, 1980) which has been shown to be of great value in the surgical treatment of teat injuries in the standing animal.

As early as 1908, BIER reported a similar method of anesthesia in the extremities of humans. He interrupted the blood supply proximal and distal to the surgical site with a tourniquet and injected a local anesthetic into a subcutaneus vein. Within 5 minutes complete anesthesia was achieved. ANTALOWSKY (1965) modified this method for use in claw surgery in cattle and was able to achieve anesthesia for up to 120 minutes. Many authors have shown equally good results and this method of local anesthesia is now widely used for surgery of extremities in cattle (SURBORG, 1984).

The following method has been used successfully in the intravenous anesthesia of the teat: The animal is sedated with 1 ml Xylaxine ® administered intravenously and a surgical scrub is applied to the teat area. Oxytocin (30 IU intravenously) is administered simultaneously and any secretion from the injured mammary gland is removed through a canula. The secretion can then be examined. A Doyen intestinal forceps is used at the base of the teat to interrupt any blood supply. Using a small gauge needle (0.9 mm diameter), a blind puncture is made tangentially towards the teat cistern into one of the many paracisternal located veins. The correct location of the needle is indicated by the efflux of blood (Fig 1). Eight to 10 ml of a 2%local anesthetic are then administrated. After a short second disinfection of the area, surgery can be started. If a teat amputation is performed, antibiotics are injected into the mammary gland before the forceps are applied.

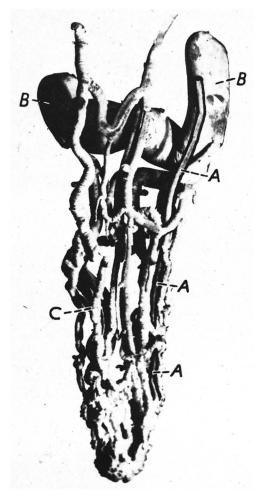
The intravenous application of the local anesthetic did not lead to any adverse reaction in over 100 surgeries and also did not seem to influence wound healing. The use of local intravenous anesthesia in teat surgery of cattle has the advantage that usually only one injection is necessary and only a small amount of anesthetic needs to be used to achieve complete anesthesia.

Difficulties might occur if the patient moves during the injection and if the injuries are fresh. In these cases, larger blood vessels are not sealed and the anesthetic will exude from the wound. This method of local anesthesia also cannot be used if the injury lies proximal to the base of the teat.

Fig 1. Blind puncture of a teat vein with a disposable needle (0.9 mm diameter). Correct localization is seen by the efflux of blood. A Doyen intestinal forceps interrupts the blood supply to the injured teat.



Fig 2. Plastoid of the teat vasculature of a cow. (From: Le Roux and Wilkens, 1959). A: A. papillaris; B: Parts of the Furstenberg's venous ring; C: Veins of the vascular layer Rieder.



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Fig 3. Venography of a teat. Ten ml of a radiographic dye were injected into a subcutaneously located vein to visualize the vasculature.

