Epidemiological Investigations of Bovine Protozoal Abortion in California Dairy Herds

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Introduction

Bovine protozoal abortion (BPA) is a newly recognized fetal infection caused by a *Neospora*-like protozoan parasite. Aborted fetuses have characteristic histologic lesions of focal nonsuppurative necrotizing encephalitis, nonsuppurative myocarditis and myositis. The diagnosis is confirmed by immunohistochemistry using antisera to Neospora caninum on the fetal tissues^{6,11}. Neospora-like protozoa abortions were first reported from an abortion storm in a dairy in New Mexico¹⁴ and are now recognized as an important, widely distributed cause of abortion ^{1.} 5,8,9,12-14. The infection has been identified in 413 fetal samples submitted to the California Veterinary Diagnostic Laboratory for immunohistochemistry. These samples represented 224 herds in 10 states in the United States as well as Mexico and Canada The infection is common in California where it is the most frequently diagnosed cause of bovine abortion^{2,5}. In this study, data from bovine abortion submissions to the California Veterinary Diagnostic Laboratory (CVDL) from the San Joaquin Valley over a 6 year period are examined. The objectives were to determine the prevalence rate and temporal and seasonal patterns of occurrence of BPA.

Methods

Data used included results from fetuses submitted for diagnosis to the CVDL at Tulare, California. This included records from all fetuses submitted from dairies in an eight county area from January 1, 1985 to December 31, 1990. Counties included were San Joaquin, Stanislaus, Merced, Madera, Fresno, Kings, Tulare and Kern counties, which are located in the southern portion of the Central Valley of California. Data files included such information as diagnosis, estimated fetal age, animal information, clinical history, client and veterinarian. R:base computer files were joined with auxiliary data files and applications to form the database used in the study. Auxiliary files included results of Neospora caninum immunohistochemistry, weather data, and dairy herd population information from California County Farm Advisors Diagnoses in each fetus were reviewed and summarized. offices. Retrospective immunohistochemical testing was performed if fetal lesions were consistent with a protozoal infection^{2,5,11}. Fetuses with typical lesions and positive immunohistochemistry results were identified as BPA and considered to be infected with the Neospora-like protozoa. Data analysis was completed using BMBP Statistical Software and Statgraphics Secular trend in BPA was assessed using weighted regression of 3.0. transformed monthly rates of BPA. Seasonality was assessed by comparison between the 12 month cumulative distribution of monthly submissions of BPA and the 12 month cumulative distribution of fetuses at risk of aborting during each month. The distribution of fetuses at risk of abortion was estimated using data from 33,000 pregnancies of cows on dairies in Tulare county during the same 6 year period. The monthly cumulative abortions among cows 4 to 7 months pregnant were compared to the cumulative monthly proportion of BPA.

Results

A total of 698 fetuses were submitted to the CVDLS-Tulare during the study period (table 1). A definitive diagnosis as to the cause of abortion was obtained in 323 fetuses (46.3%). The largest etiologic group was BPA (170 fetuses, 24.4\%), which was more frequent than all other causes of abortion combined (153 fetuses, 21.9%). An additional 47 fetuses (6.7%) had lesions suggestive of fetal protozoal infection, protozoal although infection was not confirmed а by immunohistochemistry. Estimated gestational ages available in 691 cases ranged from 1 to 9 months, with a mean of 6.0 months. BPA fetuses had a gestational age range from 3 to 8 months, with a mean of 5.3 months. Most (78%) of the BPA fetuses were aborted at 4 to 6 months gestational age.

Herd prevalence rate was estimated to be 33.1% (table 2). A total of 311 dairies submitted 1 or more fetuses for diagnosis and 103 herds had at least 1 BPA. Herd prevalence rate was similar for all counties. The prevalence rate was higher among herds that submitted more than one fetus. The estimated prevalence rate among the 162 herds that submitted a single fetus was 14.8%. Herds submitting 2 fetuses (65 herds) had a 52% prevalence rate. Herds submitting 3 to 6 fetuses (71 herds) had a 61% prevalence rate. All herds submitting more than 6 fetuses had at least one BPA case.

The temporal distribution of BPA cases was variable among the 47 herds that submitted 2 or more BPA cases. In 23 of these herds, BPA submissions occurred during a single episode of abortions on the dairy. However, in the remaining 24 herds the cases occurred over a considerable span of time ranging from 2 to 42 months between submission of the first and last BPA cases from that herd. The clinical histories that accompanied the fetal submissions were often incomplete so a retrospective estimation of the herd abortion rate at the time of the submission was not possible. However, in a few herds with a BPA diagnosis, explosive epidemics of abortion involving 5% to 8% of pregnant cattle were reported.

The results of regression analysis on proportion of fetuses diagnosed with BPA indicated no secular trend of significant increase or decrease during the 6 year period (p=0.22). Cumulative monthly total of BPA cases and total submissions indicated that BPA cases occurred throughout the year. The proportion of submissions that were BPA ranged from 7% in September to 33% in May. Two periods of greatest BPA proportion occurred during April to June and November to January. A significant seasonal difference was observed between the 12 month cumulative distribution of monthly submissions of BPA and the 12 month cumulative distribution of fetuses at risk of aborting during each month. During the months of November to February there was an 16% increase in BPA over the expected number based on the estimated number of cows in the 4th to 7th month of pregnancy at risk of abortion during those months.

The San Joaquin Valley region has a large dairy cattle population of approximately 710,000 head. The average herd sizes in the counties ranged from 350 to 1000 cows. The dairies are all drylot design with no pasture. In most dairies, housing is minimal and shades are provided in the pens. A small percentage of herds have free stall barns. The typical ration utilizes alfalfa hay as the major roughage component, which may be fed separately or mixed with other feed. Corn silage is fed in most dairies. Dry cow rations include oat hay. The most commonly fed grains are steamed and rolled corn and barley. Common byproducts included in the rations include whole cottonseed, cottonseed meal and beet pulp. A variety of other by-products are fed depending on price and availability. Rations are fed as a total or partial mix in bunks or feed aprons adjacent the pens. Feeding in the parlor is limited. Vaccination programs vary, but most herds have some regular vaccination program for BVD, IBR, PI3 and leptospirosis. San Joaquin Valley is a semi-arid region, which receives most of its annual rainfall in the winter months (November to March). The annual mean rainfall was 6.1 to 9.4 during the 6 year period. The winters are mild with monthly mean low temperatures of 35 to $40^{0}F$. Summers months are hot and dry with monthly mean high temperatures of 90 to 95°F.

Discussion

Previous studies have shown that *Neospora*-like protozoal infections were associated with a significant proportion of bovine abortions in California^{2,5}. In this study, the proportion of fetuses with *Neospora*-like protozoal infection was 24.4%, which is greater than the previous reports. This higher proportion of BPA is thought to reflect the fact that all submissions were from drylot dairies. We focused on dairies in San Joaquin Valley because CVDL data were available since 1985. This allowed us to look for trends in the proportional rate, herd prevalence, and seasonal influences in the occurrence of BPA.

The results indicate that BPA is not a new disease in the San Joaquin valley. There was no significant change in the proportional rate of occurrence of BPA over the 6 year period. This stability in occurrence suggests that although it was not recognized, BPA was present before 1985. It is possible that BPA may be a factor in the relatively high estimate of 7 to 14% for the "normal" or "expected" abortion rate in large California style drylot dairies^{10,15}.

The high proportion of BPA diagnoses observed in the CVDL submissions reflects a widely distributed infection in large numbers of dairies and is not the result of skewed sampling of a limited number of herds. The herd prevalence of BPA infection was estimated to be 33.1%. This is thought to be an underestimation due to the low number of fetuses submitted from most herds. As more abortions were sampled on a dairy the likelihood of a BPA diagnosis in that herd was increased. The herd prevalence rate and proportional rate was similar throughout the 8 county area indicating that BPA is widespread.

In some herds the disease was associated with explosive epidemics in which 5% to 8% of pregnant cows aborted in a few weeks. However, in herds in which multiple BPA fetuses were submitted, cases occurred over a period of months to years indicating that the problem persists in the herd. It has yet to be determined whether this represents new infections, introduction of infected animals, or reinfection of persistent infection of the same animal.

While BPA occurred throughout the year, there was a period from November to February in which cattle were at an increased risk of abortion due 0 The life cycle and source of infection of the Neospora-like protozoa is not known so we can only speculate about the significance of seasonal influences on BPA. Toxoplasma gondii is a similar organism which suggests that the BPA agent may have a life-cycle involving a carnivore as the definitive host. In this hypothesis, this unidentified host would have an intestinal infection resulting in the shedding of oocysts in the feces. It would reasonable that cattle could then be infected by ingestion of feeds contaminated with feces from this definitive host. It is possible that the cooler temperatures or higher moisture found during this time in the San Joaquin Valley might improve viability of oocysts in the environment. Alternate explanations would include seasonal changes in the population or behavior of definitive hosts on the dairy or changes in feeds that might contain the contaminating oocysts. In conclusion, BPA is a major cause of abortion in drylot dairies in the San Joaquin Valley. The infection is widespread and there is a minimum estimated prevalence rate of 33.1%. The fact that the

proportion of fetuses diagnosed with this infection has remained relatively stable since 1985 suggests that it is not a new disease in the area. While BPA occurs throughout the year, there is an apparent increased risk of BPA in the months of November, December, January, and Further investigations are needed to identify the risk February. factors that are associated with the infection. A prospective study is underway on infected and presumed noninfected dairies to sample all aborted fetuses and to analyze the feeding, management and environmental Information about risk factors that may contribute to infection. factors and possible sources of the infection will provide a basis for establishing effective methods of prevention and control.

Legends:

to BPA.

Table 1: Summary of diagnoses of 698 aborted fetuses submitted to the California Veterinary Diagnostic Laboratory at Tulare from January 1, 1985 to December 31, 1990.

Diagnostic Category	No.	% total
Cause identified	323	46.3%
<u>Neospora-like protozoa</u>	170	24.4%
Bacteria	101	14.5%
Viruses	30	4.3%
Mycotic	5	0.7%
Other protozoa	2	0.3%
Epizootic bovine abortion	10	1.4%
Non-infectious causes	5	0.7%
Lesions present, no cause identified		
Suspect protozoa infection	47	6.7%
All other lesions	150	21.5%
Cause not identified, no lesions	178	25.5%

	Abortions			Herds		
County	Total	BPA	%BPA	Total	BPA	% Infected
San Joaquin	9	3	33%	5	2	40%
Stanislaus	131	34	26%	68	24	35%
Merced	89	16	18%	52	12	23%
Madera	17	5	298	6	2	33%
Fresno	82	23	28%	36	13	36%
Kings	100	19	19%	39	12	31%
Tulare	240	62	26%	90	35	39%
Kern	30	8	278	15	3	20%
Total	698	170	24.4%	311	103	33.1%

Table 2: San Joaquin Valley herds with a BPA diagnosis: CVDLS Tulare Laboratory submissions (1/1/85 to 12/31/90)

References

1. Anderson ML, Blanchard PC, Barr BC, Hoffman R: A survey of causes of bovine abortion occurring in San Joaquin Valley, California. J Vet Diag Invest 2:283-287,1990. 2. Anderson ML, Blanchard PC, Barr BC, Dubey JP, Hoffman R, Conrad PA: *Neospora*-like protozoal infection as a major cause of abortion in California dairy cattle. J Am Vet Med Assoc, 198:241-244, 1991. 3. Barr BC, Anderson ML, Blanchard PC, Daft BM, Kinde H, Conrad PA: Neospora-like protozoal infections: A two year retrospective study of cases in California. Vet Pdt McAssoc, 198:241-244, 1991. 3. Barr BC, Conrad PA, Dubey JP, Anderson ML: *Neospora*-like encephalitis in a calf: pathology, ultrastructure, and immunoreactivity. J Vet Diag Invest 3:39-46, 1990. 5. Barr BC, Anderson ML, Dubey JP, Conrad PA. *Neospora*-like protozoal infections associated with bovine abortions. Vet Pathol 28:110-116, 1991. 6. Dubey JP, Hattel AL, Lindsey DS, Topper MJ: Neonatal *Neospora caninum* infection in dogs: Isolation of the causative agent and experimental transmission. J Am Vet Med Assoc 193:1259-1263, 1988. 7. Dubey JP, Carpenter JL, Speer CA, <u>et al</u>: Newly recognized fatal protozoan disease of dogs. J Am Vet Med Assoc 192:1269-1285, 1988. 8. Dubey JP, Leathers CW, Lindsey DS: *Neospora caninum*-like protozoan associated with fatal myelitis in newborn calves. J Parasitol 75:146-148, 1989. 9. Dubey JP, Miller S, Lindsay DS, Topper MJ: *Neospora caninum*-associated myocarditis and neophalitis ans aborted calf. J Vet Diagn Invest 2:66-69, 1990. 10. Klingborg DJ: Normal reproductive parameters in large "California-style" dairies. , Veterinary Clinics of North America: Food Animal Practice vol. 3, No. 3, pp.483-499, 1981. 11. Lindsey DS, Dubey JP: Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. Am J Vet Res 50:1983-9, 1983. 12. Parish SM, Maag-Miller L, Besser TE, Weidner JP, McElwain T, Knowles DP, Leathers CW: Myelitis associated with protozoal infection in newborn calves. J Am Vet Med Assoc 191:1559-1600, 1987. 13. Shivaprasad HL, E

Summary

Bovine protozoal abortion (BPA) is a newly recognized fetal infection caused by a *Neospora*-like protozoan parasite. In the drylot dairies of the San Joaquin Valley of California, BPA is the major identified cause of abortion, seen in 24.4% of aborted fetuses submitted to the diagnostic laboratory. The proportional rate of diagnosis has been stable since 1985 suggesting that BPA is not a new disease in the region. The infection is widespread among the dairies with a minimum estimated herd prevalence rate of 33.1%. While BPA occurs throughout the year, there is an increased risk of BPA in the months of November, December, January and February.