Therapy for Johne's Disease

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Introduction

Johne's disease is becoming recognized as one of the most important infectious diseases of the cattle population in the United States. Prevalence studies indicate a nationwide incidence of nearly 3%[1] in dairy cattle while regional studies reveal infection rates of 10.8[2] to 18%[3] in some northeastern and north central states. The prevalence varies with the type of cattle (dairy vs beef) breed, and geographic area. Regulations regarding the management of infected herds and individuals vary from state to state, but control measures based on periodic testing and slaughter of infected animals should be implemented in most instances. However, some cattle are exceedingly valuable and such measures can represent a considerable economic loss. Often owners of such animals will request that all possible measures be taken to harvest the full genetic potential of the animal. Thus veterinarians are sometimes requested to treat an animal with Johne's disease. Before any treatment protocol is initiated, several factors should be discussed with the owners including possible transmission of *Mycobacterium paratuberculosis,* potential public health concerns, and the effects of therapeutic agents on reproductive capabilities.

Potential for Transmission of *M. paratuberculosis* Infection

There is some reason for concern in using semen from bulls infected with *M. paratuberculosis.* Organisms have been cultured from the semen and accessory sex glands of infected bulls.[4] The organism has been demonstrated to survive freezing and semen processing procedures similar to those used by many commercial bull studs, but antibiotics and extenders may eliminate or diminish the viability of the organism in the processed semen.[5] The number of organisms found in the semen of infected bulls has been exceedingly low and present in only periodic ejaculates.[4,6] Attempts to establish infection via the intrauterine route have not been successful except when extremely large quantities of the organism were used.

The potential for *in utero* transmission is quite high as approximately 1/4 of infected cows have been shown to have culture positive fetuses.[7] The time of gestation when fetal infection occurs and by what mechanism the organism enters the fetus is unknown, but the potential for transmission of infection to the fetus is substantial over the full gestation. However, most infected cows undergoing treatment for Johne's disease would likely be utilized for embryo production in which the time of contact of the embryo with the infected cow is minimized. It has been demonstrated that organisms can be recovered from the uterus of cows with natural infection.[8,9] Recent *in vitro* and *in vivo* experiments indicate that organisms may be present in the flush fluids of infected cows during embryo transfer.[10] Furthermore, embryos have been found to attract *M. paratuberculosis* organisms to their surface. There is limited information to indicate the risk that this small number of organisms clinging to the wall of the embryo represents toward infection of the recipient animal or the resulting pregnancy. In light of other evidence, it would seem that the risk of infection is extremely low under these circumstances. Finally, animals with clinical Johne's Disease are known to excrete large numbers of organisms in their feces.[11]

Treatment protocols to date have not been effective in eliminating fecal shedding of the organism.[12-14] Thus, unless the animals are carefully isolated and stringent measures adopted for sanitation removal of infective feces, significant risk persists for infection of other animals on the same farm or handled by personnel caring for the infected animal.

Public Health Concerns

Most of the drugs used in the treatment of paratuberculosis are not approved for use in cattle, so little data exists on tissue and milk residues for these drugs. The drugs are know to have the potential for adverse effects in humans[15] and without accurate withholding time guidelines it is safest to consider meat and milk from treated animals unfit for human consumption. The high value of these animals for genetic production should make forfeiture of slaughter and milk revenues acceptable for the owners.

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Effects of Drugs on Fertility

There is little published information regarding the effects of drugs on semen and embryos. Both clofazamine and isoniazid have been shown to have feticidal effects in certain laboratory animals and are not recommended for use during pregnancy in humans.[15] These effects are reported at very high dosages but with the brief period of exposure encountered with embryo transfer, this may not pose a significant risk. The effects on semen quality and viability are less well known. Our clinical experience with treated bulls has revealed that viable semen can be collected during the treatment period.

Specific Drugs Used in Therapy

Many of the reported clinical trials on treatment of cattle with Johne's Disease are based on knowledge of treating man and animals with other mycobacterial disease such as tuberculosis and leprosy.[16] Some basic concepts of mycobacterial therapy include that the drugs must be able to penetrate into cells since the organisms are intracellular pathogens. Multiple drug combinations are frequently used in treating tuberculous patients and this may be necessary in the treatment of Johne's Disease as well. Long-term therapy is often required to achieve remission and prevent or lessen the severity of relapses.[12,13]

It must be emphasized that treated animals should be considered to remain infected throughout the treatment period and that they are likely to continue to excrete organisms in the feces even if in smaller quantities. A number of chemotherapeutic agents have been used experimentally and clinically on infected cattle. These include clofazamine, isoniazid, rifampin and streptomycin.[12,13,17-20]

Case No. 1

A 5-year-old Angus cow was used as an embryo donor. Diarrhea developed acutely and persisted for more than one week. She ws admitted to The Ohio State University Veterinary Teaching Hospital (VTH) for diagnosis and treatment at that time. The cow was depressed and febrile (T - 104°F). She had diarrhea that was very watery with no evidence of bloody mucus or mucosal shreds. Dehydration was moderate and she refused feed. Diagnostic testing for Salmonellosis was conducted because of signs of diarrhea, depression, and fever. Testing for Johne's Disease was conducted because diarrhea persisted for an extended period of time and no blood, mucus, or mucosal shreds were observed in her feces.

Her diagnostic test results were as follows:

Rectal Mucosa Biposy

	Hospital day	Result
Acid Fast Stain and direct microscopic examination	1	negative
	7	positive
Histopathology	7	chronic lymphoplasmocytic proctitis

Salmonella Cultures

Hospital Day	Cultures Results
1	neg.
2	neg.
3	pos.
4	pos.
5	pos.
5 mo.	neg.

Serologic testing for Johne's Disease during the first week revealed positive results on both the AGID and ELISA test.

Although the diagnostic test results were mixed, we considered the primary diagnosis to be Johne's Disease with concurrent Salmonellosis. The fact that the first two cultures were negative but subsequent cultures were positive illustrates the importance of following the recommendation of obtaining al least five fecal cultures before the animal is considered negative. In this cow salmonella was considered to be a secondary invader.

Therapy

Therapy consisted of products targeted at both *M. paratuberculosis* and Salmonella organisms. Rifampin was given orally at a dosage of 10 mg/lb and was continued for 28 days. Gentamicin was given IM at 1 mg/lb BID for seven days. Feces became nearly normal after about two days of therapy.

Isoniazid was given orally at the dosage of 5 mg/lb daily. Therapy was instituted on day 3 after admission and was continued on the basis of three weeks on treatment followed by one week off treatment. Treatment was continued on this schedule for 760 days.

In summary, initial therapy consisted of a combination of drugs and was targeted at bringing acute salmonellosis and Johne's Disease under control. Thereafter, she was maintained exclusively on Isoniazid.

Rectal mucosa biopsy and fecal culture for *M. paratuberculosis* were performed approximately every six months for a total of four times. Both tests were positive at all four testing times. This experience illustrates that the treatments used on this cow did not eliminate *M. paratuberculosis* infection or the lesions.

Hemograms and Diagnostics

		Time after treatment			
Test	Initial	6 mo.	12 mo.	18 mo.	24 mo.
fecal culture	pos		pos		pos
rectal mucosa biopsy and acid fast stain	pos		pos		pos
Total protein (gm/100ml)	4.5	6.0	7.2	5.8	
Total WBC count	2400	4500	5400	3000	

The total protein was very low (4.5 gm) initially and increased as clinical signs abated after treatment.

The low initial white blood cell count was accompanied by a degenerative left shift thought to be due to Salmonellosis. Although on subsequent hemograms she continued to have low total cell count, the differential cell count was normal.

Embryo Transfer Results

Time after Treatment Initiation	Embryos Transferred	Pregnancies	Normal Calves Delivered
9 mo.	7	4	4
11 mo.	1	1	1
12 mo.	2	2	2
14 mo.	2	2	2
TOTAL	12	9	9

Nine calves resulted from embryos produced and transferred during the first 14 months after treatment was initiated. During the following year embryo transfer was attempted five times but no embryos were of sufficient quality for transfer. During this period she had intermittent diarrhea and weight loss despite continued treatment. She had a loss of weight and her general body condition was probably inadequate to support normal embryo production. It should be noted that the nine embryos resulting in normal calves were all produced while the cow was receiving isoniazid.

The cow died of diarrhea and weight loss approximately 26 months after treatment was instituted. A necropsy could not be performed.

Case No. 2

A 7-year-old Holstein cow was a resident donor at an embryo transfer facility. She developed acute abdominal pain and was referred to the OSU-VTH. During examination she was found to have an elevated heart rate (96/min), a normal temperature (102.6°F) and an area of tympanic resonance in the right paralumbar fossa. Submandibular edema and diarrhea were also noted.

An exploratory laparotomy was performed to determine the cause of pain. She was found to have cecal dilatation and firm adhesions between the liver and body wall. Because diarrhea had been observed, a biopsy of the ileocecal lymph node was obtained. The biopsy revealed granulomatous lymphadenopathy but no organisms were observed.

A rectal mucosal biopsy was subsequently performed which was positive for acid fast organisms. At this time a tentative diagnosis of Johne's Disease was made and treatment was initiated. Additional diagnostics were also performed as follows:

Fecal culture - initally positive for *Mycobacterium avium.* Subsequent fecal cultures were positive for *Mycobacterium paratuberculosis.*

AGID - initially two tests one month apart were negative. Subsequent tests were positive.

ELISA - numerous tests at intervals throughout the clinical course remained weakly positive. Salmonella cultures - five consecutive daily cultures were negative.

The total protein and albumin were both low (TP 4.8 gm, Alb 1.6 gm) and accounted for the mandibular edema.

Treatment

Initially Clofazamine was given at 1 gm/day. Therapy was discontinued after 10 days because diarrhea seemed to worsen and her appetite decreased.

She was switched to rifampin at 10 mg/lb/day, ampicillin at 5 mg/lb/day and isoniazid at 6/mg/lb/day. Her general condition immediately improved and diarrhea subsided after about one week on this regimen. After two weeks, rifampin and ampicillin were discontinued and she was maintained on isoniazid at 5/mg/lb/day. Isoniazid was given for three weeks followed by one week off treatment. The one week off treatment in both cases was done to reduce the potential for hepatotoxicity sometimes attributable to isoniazid in man. Whether this practice is helpful or necessary in cattle is unknown.

Treatment continued for over 2 years and she has remained in remission for nearly the entire time. Periodically she relapsed with diarrhea, mandibular edema and weight loss. Supportive treatment with fluids and antibiotics was given during these episodes.

Embryo Transfer Results

Time after Treatment Initiation	Embryos Collected	Embryos Transferred	Pregnancies
3 mo.	18	15	11
4 mo.	24	2	1
6 mo.	1	0	0
7 mo.	9	6	0
8 mo.	11	1	1
10 mo.	15	2	1
11 mo.	1	1	1
12 mo.	25	12	8
15 mo.	5	0	0
17 mo.	2	0	0
22 mo.	0	0	0
23 mo.	21	6	4
25 mo.	0	0	0
TOTAL	132	45	27

The first several successful embryo transfers were performed while the cow was receiving treatment. To reduce the possibility of drug interference with embryo viability,

all embryo transfers after 12 months were performed while the cow was off therapy. Although some viable embryos were collected during this period, it is not possible to determine if viability relates to lack of treatment or other factors such as general body condition.

At this time the cow is still receiving treatment and still in remission although she has periodic relapses. She has been positive on fecal culture for *Mycobacterium paratuberculosis* conducted periodically throughout the clinical course.

Summary

Several previous reports have described successful treatment of Johne's Disease but few, if any, have described successful embryo transfer during treatment. These cases illustrate that viable embryos could be transferred from cows while receiving isoniazid for the treatment of clinical Johne's Disease. Embryos were collected multiple times and resulted in normal calves being delivered. It is not known if any of the calves are infected but the probability of infection is low and all remained healthy.

Treatment of these cows did not eliminate infection or lesions as evidenced by consistently positive fecal cultures and biopsies during treatment and the periodic relapses.

Whether treatment was cost effective or not depends on the value of the progeny resulting from embryo transfers. Both owners have indicated they feel the effort was worthwhile.

The cost of treatment is illustrated on the following table which assumes the dosages listed and prices of drugs at the time these cows were treated.

Cost of Treatment for Johne's Disease

Drug	Dose/day	Cost	Cost for a 1,000 lb cow
Isoniazid	5 mb/lb	$.04/gm	$.2/day
Clofazamine	5 mg/lb	1.71/gm	8.55/day
Rifampin	10 mg/lb	4.10/gm	41.00/day
Gentamicin	2 mg/lb	4.00/gm	8.00/day

The primary drug selected in these cows was isoniazid. It was selected because it is apparently safe and effective for long-term therapy and has minimal cost. Clofazamine has also been used for long-term treatment but one of these cows did not seem to respond favorably to it. Rifampin was used initially to aid in countering acute disease and achieving remission. It was discontinued as soon as possible due to the high cost of of the drug. In both animals it was given in conjunction with another antibiotic to reduce the possibility of organism resistance. Whether *M. paratuberculosis* readily become resistant to rifampin is unknown. In one cow gentamicin was given. It was chosen to treat the Salmonellosis which was a concurrent and perhaps secondary infection. One of these cows also had *M. avian* cultured from feces. It is likely that this organism was also a secondary invader.

Since none of the drugs used and described are approved for cattle, owners were advised at the outset that milk could not be marketed and the cows could never be slaughtered. Both owners readily agreed with this requirement because the revenue potentially derived from embryos far surpassed any comparatively small income which could be generated from the sale of milk or slaughter.

Neither of these cows eliminated the infection and shed organisms at least periodically throughout the treatment period. Therefore they were kept in isolation at the OSU-VTH during the entire time. It is very important to consider the potential for transmission of infection throughout a herd from infected cows via feces. Extra ordinary isolation facilities are required to minimize this risk since the treatment period is so long. It is very difficult to achieve adequate isolation in a farm setting.

References

1. Merkal RS, Whipple DL, et al. Prevalence of mycobacterium paratuberculosis in ileocecal lymph nodes of cattle culled in the United States. JAVMA 1987;190:676-679 2. Arnoldi JM, Hurley SS, Lesar S. Johne's disease in Wisconsin cattle, in Proceedings. Internat Colloq Res Paratuberculosis 1983; pp 16-21. 3. Chiodini RJ, Van Kruiningen HJ. The prevalence of paratuberculosis in culled New England cattle. Cornell Vet 1986;76:91-104. 4. Larsen AB, Stalheim OHV, Hughes DE, et al. Mycobacterium paratuberculosis in the semen and genital organs of a semen donor bull. JAVMA 1981;179:169-171. 5. Larsen AB, Kopecky KE. Mycobacterium paratuberculosis in reproductive organs and semen of bulls. AJVR 1970;31:255-258. 6. Hoffsis GF, Davis GW, Monke DR. The sensitivity of culture in determining the number of Mycobacterium paratuberculosis organisms present in experimentally inoculated bovine semen. In Proceedings. Second Internat Colloq on Paratuberculosis 1988; pp 265-276. 7. Seitz SE, Heider LE, Hueston WD, et al. Bovine fetal infection with Mycobacterium paratuberculosis. JAVMA 1989;194:1423-1426. 8. Kopecky KE, Larsen AB, Merkal RS. Uterine infection in bovine paratuberculosis. AJVR 1967;28:1043-1045. 9. Pearson JKL, McClelland TG. Uterine infection and congenital Johne's disease in cattle. Vet Rec 1955;67:615-616. 10. Rohde RF. The potential for transmission of Mycobacterium paratuberculosis through embryo transfer. MS Thesis, Ohio State University, 1988. 11. Chiodini RJ, Van Kruiningen HJ, Merkal RS. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 1984;74:218-262. 12. Merkal RS, Larsen AB. Clofazimine treatment of cows naturally infected with Mycobacterium paratuberculosis. AJVR 1973;34:27-28. 13. Whitlock RH, Divers T, et al. Johne's disease: a case study with clofazimine therapy in a dairy cow. in Proceedings Intern Colloq Res Paratuberculosis 1983; pp 231-237. 14. Gilmour NJL. Studies on the effect of the rimino phenazine B663 (G. 30320) on Mycobacterium johnei. Br Vet J 1966;122:517-521. 15. Physicians Desk Reference. 44 Edition, 1990; Medical Economics Co Inc, Publisher, Oradell NJ, pp 856,865. 16. Goldberger MJ. Antituberculosis agents. Med Clinics N Amer 1988;72:661-668. 17. Larsen AB, Vardaman TH. The effect of isonicotinic acid hydrazide on Mycobacterium paratuberculosis. JAVMA 1953;122:309-310. 18. Rankin JD. Isoniazid: Its effect on Mycobacterium johnei in vitro and its failure to cure clinical Johne's disease in cattle. Vet Rec 1953;65:649-651. 19. Baldwin EW. Isoniazid therapy in two cases of Johne's disease. VM/SAC 1976;71:1359-1362. 20. Slocombe RF. Combined streptomycin-isoniazid-rifampin therapy in the treatment of Johne's disease in a goat Can Vet J 1982;23:160-163.