COMPARISON OF *FUSOBACTERIUM NECROPHORUM* ISOLATES FROM LIVER ABSCESSES, RUMINAL WALLS, AND RUMINAL CONTENTS OF FEEDLOT CATTLE [1]

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Summary

DNA finger printing (restriction fragment length polymorphism analysis) was employed to genetically compare *Fusobacterium necrophorum* isolates of liver abscesses, ruminal wall, and ruminal contents from the same animal. *Fusobacterium necrophorum* isolates from liver abscesses were genetically identical to the corresponding isolates from the ruminal wall in eight of nine animals tested. This genetic similarity between the isolates supports the hypothesis that *F. necrophorum* in liver abscesses originates from the ruminal wall.

(Key Words: *Fustobacterium necrophorum*, Liver Abscesses, Feedlot.)

Introduction

Liver abscesses occur most often in cattle fed high grain diets. Abscessed livers commonly are found in 10-30% of feedlot cattle at slaughter. *Fusobacterium necrophorum*, a bacterium normally present in the rumen, is the primary causative agent of liver abscesses in cattle.

The incidence of liver abscesses is generally higher in cattle with lesions of the rumen lining than in cattle with normal rumens. The positive correlation between incidence of liver abscesses and ruminal lesions was the basis for the hypothesis that *F. necrophorum* in liver abscesses is of ruminal origin. Our approach to documenting that *F. necrophorum* of liver abscesses originates in the rumen was to show that the rumen wall and liver abscess isolates are genetically identical and, thus, are progeny of a single cell.

Experimental Procedures

Samples of ruminal contents, ruminal wall tissue (from the dorsal sac), and liver abscesses were collected from 11 cattle at slaughter, packed in ice, and transported to the laboratory. Swab samples of the pus from liver abscesses, homogenates of the epithelial layer of the ruminal wall, and diluted ruminal contents were used for isolation of *F. necrophorum*.

In order to genetically compare the isolates, the technique of restriction fragment length polymorphism (RFLP) analysis of ribosomal DNA or ribotyping was employed. Ribotyping involves the fingerprinting of

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chromosomal DNA restriction fragments that contain genes coding for 16S and 23S ribosomal RNA (rRNA). Because the genes coding for rRNA are highly conserved and most bacteria contain multiple copies of rRNA genes, a reasonable number of fragments are obtained after probing, and this allows discrimination among strains within the same species.

Briefly, the procedure for ribotyping was as follows: chromosomal DNA was extracted and digested with restriction endonucleases alone (*Eco*RI, *Eco*RV, *Sal*I, and *Hae*III) or in combination (*Eco*RI and *Eco*RV).). Restriction fragments were separated by gel electrophoresis and probed with a commercially available 16 and 23S rRNA from *E. coli*. Hybridization banding patterns of the isolates were compared among isolates from all three locations within the same animal. Isolates were considered genetically different if even a single band was different between two isolates with any of the restriction enzymes used to fragment the DNA.

Results and Discussion

Out of sets of liver abscesses, ruminal walls, and ruminal content samples from 11 cattle, *F. necrophorum* was isolated from all three locations from four animals, from liver abscesses and ruminal walls in five animals, and from liver abscesses and ruminal contents from two animals. This allowed comparison of nine isolates from liver abscesses and ruminal walls and six isolates from ruminal contents and liver abscesses. The number of major bands of DNA among the Isolates ranged form nine to 11. Isolates differing by one or more bands in their hybridization patterns were considered distinct strains (Figure 1). .

The ribotypic comparison of isolates from liver abscess and ruminal wall of the ame animal showed that in eight out of nine cases, *F. necrophorum* isolated from the ruminal wall was identical to liver abscess isolates (Table 1). None of the ruminal content isolates matched with either the ruminal wall isolate or liver abscess isolate from the same animal. These results provide direct evidence for the proposed pathogenesis of liver abscesses. Prior to this, the only evidence available in support of this hypothesis was the statistical correlation between the occurrence of liver abscesses and ruminal pathology.

The genetic similarity between the isolates from liver abscesses and the isolates from the ruminal walls lends credence to the hypothesis that *F. necrophorum* of liver abscesses originates from the rumen. The lack of genetic similarity between ruminal content and liver abscess isolates is not surprising, because ruminal contents presumably have a multitude of strains. Therefore, chance is involved in finding a match. Presumably, a single strain among this multitude of strains penetrates and colonizes the ruminal wall.

Table 1. Comparison of *Fusobacterium necrophorum* Isolates from Ruminal Contents, Ruminal Wall, and Liver Abscesses

Animal	Ruminal Contents	Ruminal Wall	Liver Abscesses
1	---	✖	✔
2	---	✔	✔
3	✖	✔	✔
4	---	✔	✔
5	✖	✔	✔
6	✖	✔	✔
7	---	✔	✔
8	✖	✔	✔
9	✖	---	✔
10	---	✔	✔
11	✖	---	✔

✔Match
✖ No Match

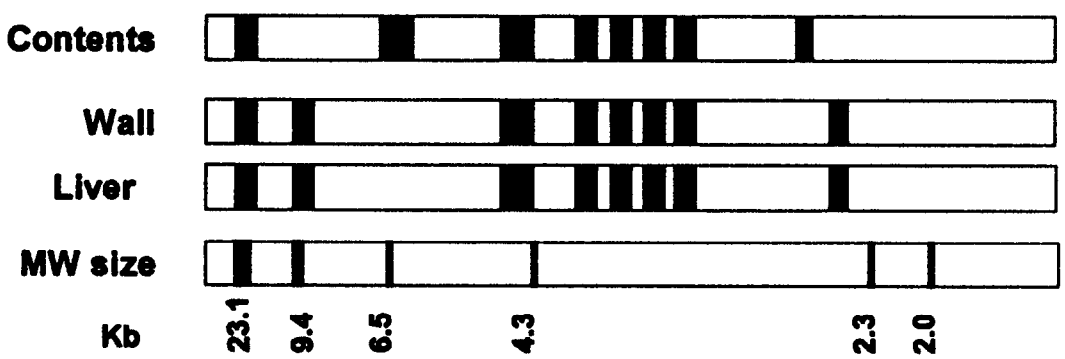

Figure 1. Hybridization Patterns of Restriction Fragments of DNA from *Fusobacterium necrophorum* Isolates from the Ruminal Contents, Ruminal Wall, and Liver Abscesses Isolated from Animal No. 6. Lane MW Has Molecular Weight Markers. *F. necrophorum* from the Liver Abscesses Was Identical to That from Rumen Wall but Different from That of Rumen Contents.