

Vertical and Horizontal Transmission of *Mycobacterium paratuberculosis*

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Abstract

Fetuses were obtained from 58 pregnant cows naturally infected with *Mycobacterium paratuberculosis* (as determined by fecal culture), but showing no clinical signs of Johne's disease. Fetal tissues (spleen, liver, kidney, ileum, mesenteric lymph node) were cultured for *M. paratuberculosis*. Only 5 of 58 fetuses were culture positive (8.6%). All 5 culture-positive fetuses were from asymptomatic cows shedding high numbers of organisms in the feces.

Milk samples were collected aseptically from 77 cows naturally infected with *M. paratuberculosis* but showing no clinical signs. Of 77 samples, 9 (11.6%) were culture positive for *M. paratuberculosis*. Supramammary lymph nodes were culture positive from 22 of 81 cows (27%).

Fetal infection and direct excretion of *M. paratuberculosis* in milk can occur in asymptomatic infected cows, but with lower frequency than cows with signs of Johne's disease.

Vertikale und horizontale Übertragung von *Mycobacterium paratuberculosis*.

Zusammenfassung

Die Feten von 58 graviden Kühen wurden untersucht. Die Kühe wiesen eine natürliche Infektion mit *Mycobacterium paratuberculosis* auf (bestimmt durch bakteriologische Untersuchung von Fäzes), zeigten jedoch keine klinischen Anzeichen von Paratuberkulose. Folgende fetalen Organe wurden zum Nachweis von *M. paratuberculosis* kultiviert: Milz, Leber, Niere, Ileum, mesenterialer Lymphknoten. Fünf der 58 (8,6 %) Feten waren bakteriologisch positiv. Die 5 bakteriologisch positiven Feten stammten von symptomlosen Kühen, die eine große Anzahl von Organismen über die Fäzes ausschieden.

Es wurden Milchproben von 77 Kühen, die eine natürliche Infektion mit *M. paratuberculosis* aufwiesen, jedoch keine klinischen Anzeichen von Paratuberkulose zeigten, untersucht. In 9 der 77 (11,7 %) Milchproben konnten *M. paratuberculosis* nachgewiesen werden. Die supramammären Lymphknoten waren bei 22 von 81 Kühen (27,2 %) bakteriologisch positiv.

Aus den Ergebnissen wird gefolgert, daß bei infizierten Kühen, die keine klinischen Symptome zeigen, eine Infektion der Feten und eine Erregerausscheidung über die Milch stattfinden kann. Die Häufigkeit der vertikalen und horizontalen Übertragung scheint niedriger zu sein, als bei Kühen mit klinischen Erscheinungen der Paratuberkulose.

Transmisión vertical y horizontal de *Mycobacterium paratuberculosis*.

Resumen

Se obtuvieron fetos de 58 vacas preñadas infectadas naturalmente con *Mycobacterium paratuberculosis* (determinado por cultivo de excremento), que no mostraban signos clínicos de enfermedad de Johne's. Se cultivaron tejidos fetales (bazo, hígado, riñón, ilion, ganglios linfáticos mesentéricos) para la identificación de *M. paratuberculosis*.

Solamente 5 fetos de 58 resultaron positivos (8.6%). Los 5 fetos que resultaron positivos fueron de vacas asintomáticas con altas descargas de organismos en el excremento.

Muestras de leche fueron colectadas asepticamente de 77 vacas infectadas naturalmente con *M. paratuberculosis* sin mostrar signos clínicos. De 77 muestras, 9 (11.6%) fueron positivas a *M. paratuberculosis*. Ganglios linfáticos supramamarios resultaron positivos en un 27% (22 de 81 vacas).

Infección fetal y excreción directa de *M. paratuberculosis* a través la leche puede ocurrir en vacas con infección asintomática, pero con menor frecuencia que vacas que muestran signos de la enfermedad de Johne's.

Paratuberculosis is a chronic granulomatous intestinal disease of ruminants caused by *Mycobacterium paratuberculosis*. Clinical signs include chronic diarrhea, weight loss, edema, and decreased milk production. However, infected cows may shed the causative organism in their feces for months to years before showing clinical signs [1,2]. Transmission is generally believed to occur when young calves ingest *M. paratuberculosis* organisms, such as when permitted to suckle an udder or exposed to other feeds contaminated with feces from an infected adult cow. Paratuberculosis control programs are designed to prevent oral-fecal transmission of the organism, by removing calves from cows immediately after birth and bottle-feeding colostrum.

However, there have been numerous accounts of transplacental transmission of the organism. In 1929, Alexejeff-Goloff reported isolating *M. paratuberculosis* from fetuses of cows with clinical signs of paratuberculosis. This was translated into English in 1935 as an abstract in the Journal of Comparative Pathology, [3], accompanied by a footnote by the editor, stating that the results "should be treated with incredulity, and regarded as the results of errors in observation." Since that time, additional individual case reports have confirmed Alexejeff-Goloff's results [4,5]. More extensive studies, involving the testing of fetuses from numerous infected (symptomatic) cows, have been published. Prevalence of fetal infection in these studies was 21%, 26%, and 37% [6-8]. However, results were not reported from infected cows not showing signs of the disease, despite the fact that the majority of infected cows in dairy herds do not manifest signs of the disease. The purpose of our study [9] was to determine the importance of fetal infection in asymptomatic but infected cows.

Paratuberculosis is widely regarded as an enteric infection, but dissemination of *M. paratuberculosis* to the udder and milk of infected cows has been reported. Direct shedding of organisms in milk (or colostrum) could reduce the efficacy of control measures which involve cleaning the outside of the udder. In his 1929 report, Alexejeff-Goloff reported the recovery of *M. paratuberculosis* from supramammary lymph nodes and milk of infected cows with clinical signs of paratuberculosis [3]. Subsequently, the organism was cultured from milk from 1 of 20 infected cows [10], and 9 of 26

[11] infected cows, all showing clinical signs. The organism has also been cultivated from udder tissue [3,12]. The above studies did not include infected but asymptomatic cows. The purpose of our studies [13] was to determine the likelihood of direct shedding of *M. paratuberculosis* in milk of infected asymptomatic cows. If prevalent, this could represent a potentially significant means for transmission of organisms to calves.

Infected asymptomatic cows were identified by fecal culture. With the cooperation of the owners and packing plant, we were able to obtain samples from these cows at the time of slaughter. We followed to slaughter 86 asymptomatic, infected cows from 25 herds. Of the 86 cows, 58 were pregnant. The fetuses were removed and brought to our laboratory for mycobacterial culture. Sterile instruments were used to remove the fetal liver, spleen, kidney, mesenteric lymph nodes, and ileocecal junction for culture. Milk samples were obtained from 77 cows, and supramammary lymph node from 81 cows. Fetal tissues, supramammary lymph nodes, and milk were cultured for *M. paratuberculosis* using Herrold's egg yolk medium. The fecal culture status of the cows was classified as "heavy" or "light" based on the number of colonies of *M. paratuberculosis* recovered on the culture tubes.

Of the 58 fetuses, 5 had at least 1 tissue sample culture-positive for *M. paratuberculosis*. The culture positive tissues included kidneys (3 fetuses), liver (2 fetuses), spleen (1 fetus), and ileum (fetus). All 5 of the infected fetuses came from cows that were heavy fecal shedders (5/28, 17%). None of the fetuses from 30 light shedders were infected.

Of the 77 milk samples, 9 (11.6%) were culture positive. Of the 9 culture positive samples, 7 were from cows classified as heavy fecal shedders (7/37, 19%), and 2 were from light shedders (2/40, 5%). Of the 81 supramammary lymph nodes, 22 (27%) were culture positive. Of the 22 culture positive samples, 18 were from heavy shedders (18/39, 46%), and 4 were from light shedders (4/42, 9.5%).

The 8.6% fetal infection rate in asymptomatic cows of this study was lower than the previous reports for symptomatic cows (21 to 37%). Our studies would suggest that dissemination to the fetus is more likely to occur in more advanced infection, since heavy shedders were more likely than light shedders to have an infected fetus. Similarly, the 19% milk infection rate in this study is lower than the 35% most recently reported for symptomatic cows [11].

On the basis of the results of these studies, we do not recommend that it is necessary to cull the daughters of all cows infected with *M. paratuberculosis*. Daughters of cows with clinical signs, or asymptomatic cows that are heavy fecal shedders should be culled. It is probably not necessary to cull daughters of light shedders, provided that post-natal transmission can be prevented.

Our studies did not include testing of colostrum samples. Presumably, colostrum samples might be infected with similar prevalence. Colostrum and milk from infected cows should not be fed to calves, or should be pasteurized before feeding.

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