Bovine Chlamydial Abortions

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Chlamydial agents are obligate intracellular pathogens of prokaryotic nature, and they comprise a large group of antigenically related and culturally, morphologically, and tinctorially similar microorganisms (1). One property characterizing chlamydial strains of several animal species is their ability to establish placental and fetal infection irrespective of the type of placentation. Chlamydial agents were first suspected as a cause of abortions in cattle in Germany in 1956 (2). Since then they were proven, by isolation from fetuses or placentas, as well as through serological evidence, to be a cause of reproductive failure of cattle (3-6). Chlamydiae were identified as a cause of bovine abortions in North America, in most countries of western and eastern Europe, in South Africa, and in some Asian countries (7-14).

Characteristics of experimentally induced chlamydial abortions will be analyzed in this contribution from clinical and pathogenetic aspects and from etiological, serological, and pathological viewpoints. In the process, the experimentally induced disease will be compared with, and related to, the more complex problem of abortion that confronts the practitioner and the diagnostician.

Are There Distinguishing Clinical Signs in Chlamydial Abortions?

Following inoculation with chlamydial agents isolated from aborted bovine fetuses, pregnant and nonpregnant cows invariably developed fever and a marked leukopenia during the next 3 to 5 days. Some also became anorectic and developed diarrhea, and a few had intermittent mucoid vaginal discharge. Adverse clinical behavior could be detected only by close examination (4, 6, 15). Predictably, clinical signs indicative of infectious events in pregnant cows were not described in herds that subsequently experienced naturally occurring chlamydial abortions (4, 5, 11, 13, 16).

Pregnant cows became infected following intravenous, intramuscular, subcutaneous, and intracutaneous inoculation at stages of gestation from 3 to 8 months. Many aborted after intravenous inoculation within shorter periods of 5 to 36 days, while those inoculated by other routes in the second and third trimester aborted or had weak calves 33 to 126 days later. Cows of all ages were susceptible (4, 6, 15, 17). Chlamydia-induced abortions were detected under field conditions as early as the fifth month of gestation, but most were found to occur later, mainly during the last trimester, toward the end of which stillborn and weak calves were also born. Chlamydial isolations were made from fetuses or placentas of naturally occurring abortions of the second trimester, but a few isolates were recovered from late-aborted fetuses as well as weak neonatal calves (4, 5, 9, 11, 17). Retained placentas were observed both in experimentally induced and naturally occurring abortions (4, 6). Chlamydial abortions occur sporadically, although in some herds as many as 20% of the pregnant cows abort. Abortions occur in beef herds in a seasonal pattern related to breeding practices, but in dairy herds they were observed throughout the year (4, 5, 16). Milk production in affected dairy cows can be greatly reduced, and sterility problems after abortions were observed in affected herds (5, 7, 16). The cows used in experimental investigations were not in milk. Recently mastitis was found to be associated with chlamydial infections in dairy herds (18).

Importance of Placental Infection and Diagnostic Implications

Chlamydemia was detected 2 to 3 days after parenteral inoculation of cattle. During this episode chlamydiae infected endometrial and chorionic sites and crossed the placenta but were soon cleared from the extrauterine organs of the dam (15). After placental passage the course of the infectious events in placenta and fetus appeared to proceed independently (4, 15, 17).

Histologic evidence revealed that chlamydial infection localized first in the endometrial epithelium of the inter- and periplacentomal areas where endometrial epithelial ulceration and direct contact with the chorion established placental infection. If the placentomes became affected during the early stage of the disease, the lesions were found in the margins. Involvement of the arcades of the placentomes was found 12 to 20 days after inoculation.

Acute inflammatory changes were evident in the maternal septal tips of the placentomes. Necrosis of the infected trophoblastic epithelium, infiltration with leukocytes, and vasculitis were observed in the chorion. Fibrinopurulent exudate accumulated between uterine and chorionic surfaces. All these microscopic placental lesions were more extensive and severe 27 to 49 days after inoculation (15, 17, 19). Cytoplasmic chlamydial inclusions were demonstrated occasionally in endometrial epithelial cells and abundantly in chorionic epithelial and trophoblastic cells of the placentas (Figures 1 and 2).

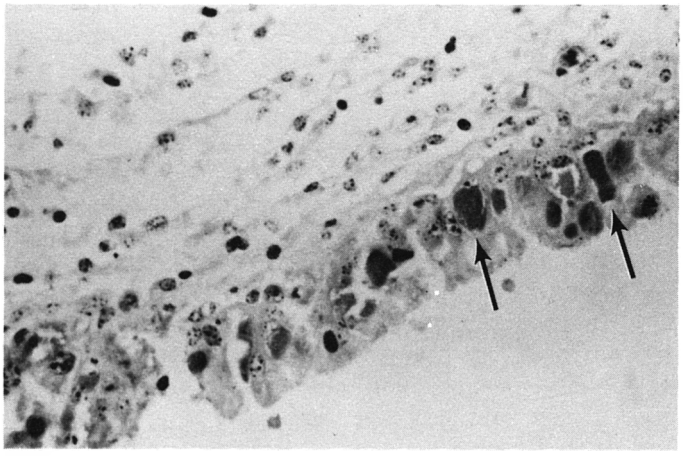

Figure 1. Chorion of periplacentomal area from a heifer 20 days after inoculation with a chlamydial strain of bovine abortion in the 2nd trimester of gestation. Numerous epithelial cells contain cytoplasmic chlamydial inclusions (arrows), Gimenez stain, X400. (Courtesy of Drs. R. P. Kwapien and S. D. Lincoln.)

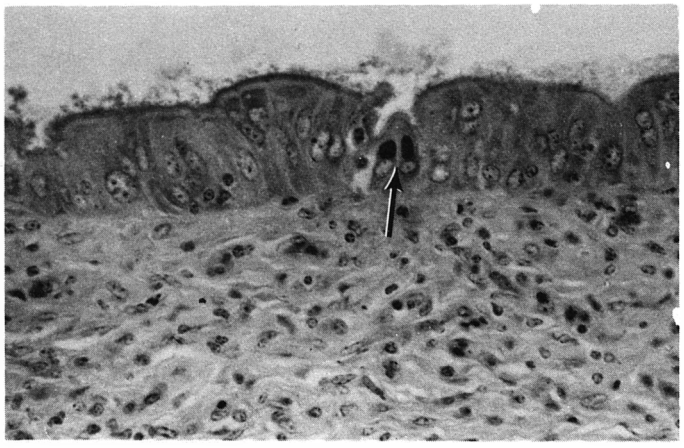

Figure 2. Endometrium of periplacentomal area of heifer from Fig. 1. Epithelial cells contain cytoplasmic chlamydial inclusions (arrows), hematoxylin-eosin stain, X400. (Courtesy of Drs. R. P. Kwapien and S. D. Lincoln.)

Striking gross lesions were found in placentas of experimental abortions, and they differed depending on the stage of gestation and the duration of infection (4, 6, 17). The cotyledons were necrotic, and the entire placenta had a brownish-yellow color when abortion occurred before the sixth month of gestation. The intercotyledonary chorion was edematous and had gelatinous consistency. Variable amounts of yellow-brown exudate were present. When cows aborted during the later stages of gestation, often only portions of the placentas had lesions, usually located in the blind placental ends. The intercotyledonary chorion in affected areas had a leathery, tough consistency and a reddish-white opaque color on the uterine surface. Affected parts of the chorion were slightly elevated and could not be scraped off. The margins of the cotyledons in affected placental areas had small foci of necrosis. Bulging edema surrounded the affected cotyledons on the fetal side of the placenta, and the chorion had an opaque, whitish color with a smooth surface (Figure 3).

Well-preserved fetal placentas from field cases of chlamydial abortions were never available to us and have virtually not been studied. Exfoliative cytologic examination of placentas from experimental cases revealed chlamydial elementary bodies after Gimenez staining, and their presence correlated well with microscopic placental lesions (4, 17). Consequently, more refined, direct cytologic examination of carefully collected placentas from field cases should enhance diagnostic opportunities. Chlamydial infection was demonstrated in a few natural cases by this method (1, 5).

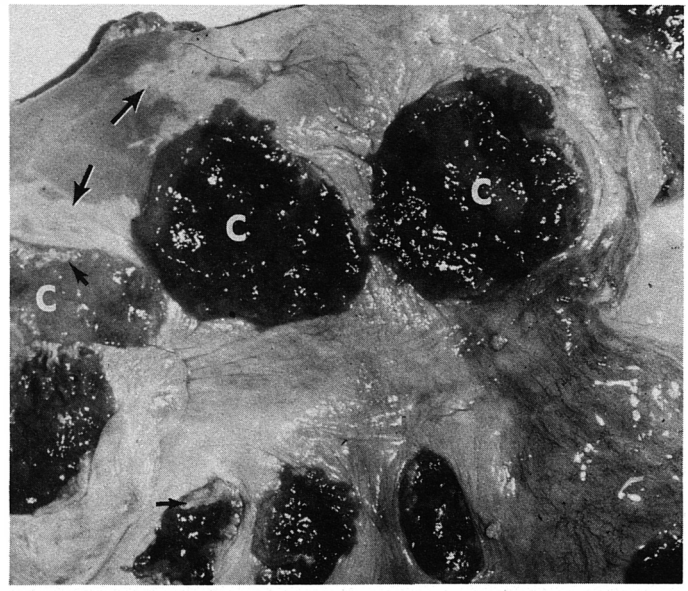

Figure 3. Placenta delivered by a cow at the end of the 8th month of gestation and 63 days after intramuscular inoculation with a chlamydial strain of bovine abortion. Arrows point towards thickened intercotyledonary chorion and to necrotic foci in the margins of cotelydons.

How Does the Fetus Respond to Chlamydial Infection?

As early as 6 days after intravenous inoculation of the dam it was possible to demonstrate infection of the fetus. Liver and spleen were among the organs first found to be infected, and with time virtually all organs may become infected. There is no single organ that is invariably involved. Infection was more difficult to demonstrate in calves that were delivered stillborn or live and weak 3 to 4 months after maternal exposure (4, 6, 19).

Changes in aborted fetuses depend clearly on the stage of gestation and duration of infection. Fetuses aborted before the sixth month of gestation have blood-tinged, subcutaneous edema and increased amounts of clear reddish pleural and peritoneal fluid. Fetuses of the third trimester of gestation where infection persisted longer than 20 to 30 days may have petechiae in thymus, mucous membranes, and the subcutis. Ascites extensive enough to cause distention of the abdomen was observed in experimental and natural cases yielding chlamydial isolates (Figure 4). The livers may be swollen, reddish-yellow with mottled appearance and a

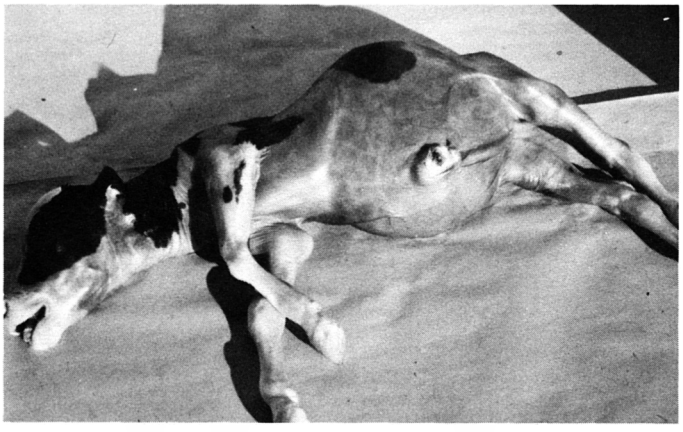

Figure 4. Bovine fetus aborted during the 7th month of gestation. Dam had been inoculated 50 days previously with a chlamydial strain of bovine abortion. The fetus had hemorrhages in the hairless skin and ascites caused distention of the abdomen.

coarsely nodular surface. This change was observed in experimentally induced infections and to variable degrees under field conditions, and it probably reflects a more chronic infection with myocardial involvement. Lymph nodes are enlarged in such fetuses (4, 6, 16, 17, 19).

Microscopic lesions were not found in fetuses examined earlier than 12 days following maternal inoculation (15). At this time hepatic and splenic lesions were detected, and after 20 days most organs were found to be involved. Necrotizing foci, sometimes with inflammatory reactions, were frequently found in liver, spleen, kidneys, heart muscle, lungs, adrenal glands, and central nervous system. These foci were midzonal in the liver, where they consisted of necrotic hepatocytes and small accumulations of monocytes and neutrophils. Phlebitis involving central, sublobar and portal veins occurred adjacent to inflammatory foci. Mononuclear cells and lymphocytes indicative of immunologic stimulation involved portal areas of the liver, renal cortex, and other organs. There was vasculitis involving liver, brain, heart and skeletal muscle, and the gastrointestinal tract. The central nervous system had inflammatory foci composed of glial elements, histiocytes, and a few neutrophils in the white matter of all levels of the brain and spinal cord. Vasculitis with perivascular cuffing was associated with these foci. There was focal, nonsuppurative meningitis (15, 17, 19). Some field cases from which chlamydiae were isolated in California had identical patterns of tissue reactions that may have been more severe (20).

How is This Infection Transmitted?

An important role in the transmission of chlamydial infections of cattle and many other animal species is played by intestinal infections and the oral entrance into new hosts (1). This mode is not yet sufficiently explored in chlamydial infections leading to bovine abortions. Intestinal epithelial cells are parasitized and mobile macrophages become productively infected. Macrophages thus may serve as a vehicle to penetrate the intestinal mucosa to establish chlamydemia (21). Pregnant heifers with intestinal chlamydial infections aborted following superinfection (15, 19). Chlamydial strains isolated from feces caused placental and fetal infection when they were given to pregnant cattle (4, 15). This mode of transmission could explain readily the incidence of this disease as it is currently recognized in most parts of the world.

Since chlamydiae were isolated from tick vectors, this enhancing and more efficient mode of transmission may well be the basis for higher rates of placental and fetal disease resulting in abortions (22, 23).

Chlamydial agents also were isolated from semen of bulls that had infections of testicle, epididymis, and accessory sex glands (24). Chlamydiae in normal semen did not interfere with fertilization, since normal embryos were recovered 3 days after insemination. However, other heifers inseminated with chlamydia-containing semen did not become pregnant, while the control heifers inseminated with the same semen free of chlamydiae conceived (25). Sexual transmission of chlamydiae in animals thus leads to sterility problems related to early embryonic death rather than placental and fetal infections. Different pathogenic mechanisms evidently are involved in chlamydial infection leading to early embryonic death or abortion.

Properties of Bovine Chlamydiae and Their Propagation

Chlamydial strains from bovine abortions were found to be antigenically and biologically indistinguishable from

strains of ovine chlamydial abortions and some strains isolated from intestinal infections of both species (1, 26). Occasional intestinal strains differed from chlamydial abortion strains, and chlamydiae associated with polyarthritis, polyserositis, and conjunctivitis can be separated from both. Chlamydial abortion strains were distinguished through their interactions with cultured cells in the presence of diethylaminoethyl dextran and cycloheximide, as well as through their growth rate and effect on the cytoskeleton of host cells (27). These investigations provided improved, highly sensitive methods for isolation and cultivation, which could be the basis for higher diagnostic efficiency if applied to clinical samples (Figure 5).

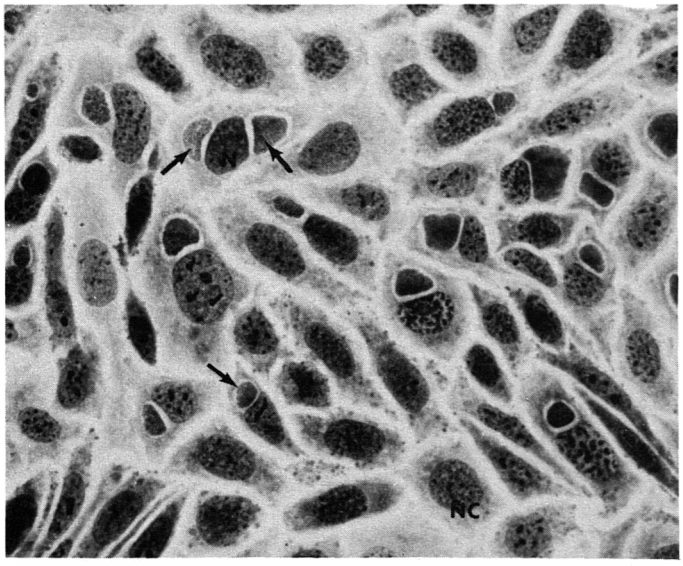

Figure 5. Cultured cells infected with a chlamydial strain of bovine abortion. Cytoplasmic chlamydial inclusions (arrows) are large and can be clearly distinguished from nuclei (N) of infected cells in this Giemsa-stained preparation. Some uninfected cells (UC) are also visible, X500.

Perspectives and Future Implications

Chlamydial abortions of cattle evidently occur worldwide in accordance with the worldwide distribution of chlamydial pathogens in the animal and human populations (1). Virtually all currently reported cases of chlamydial abortions were based on isolation and identification of the causative agent. It is now conceptually well accepted that the fastidious obligate intracellular pathogens that cause abortions are extremely difficult to isolate at the time of premature or term departure of the fetus from the uterus. They are readily inactivated in the course of intrauterine infectious events that lead to fetal death or chronic infection before expulsion of the placenta and fetus. Consequently, chlamydiae as a cause of abortion may be underdiagnosed.

The cultural isolation methods used in the past are relatively insensitive when compared with the emerging cell culture methods employing procedures to enhance chlamydial adsorption to cells, such as centrifugation and the use of DEAE-dextran, or cycloheximide to marshall cellular functions for chlamydial replication (27). Since these methods are faster and more sensitive, they should now be employed for chlamydial isolation and diagnosis.

Inoculation of pregnant cows by a variety of different routes readily leads to placental and subsequent fetal infection resulting in fetal death and abortions or the birth of stillborn or weak calves (1, 4, 6, 15, 17, 19). The initial course in placental infections evidently consists of contact infection between epithelial cells of endometrium and the chorion in peri-or interplacentomal areas. Chlamydial multiplication induced large cytoplasmic inclusions and had cytocidal consequences. The placentomes may become infected through lateral spread by contact, and the infection reaches cells in the arcades of placentomes as well as the fetus. The placental infection should be exploited diagnostically through skillful exfoliative cytology. However, suitable placental samples from field cases are seldom provided for diagnostic efforts.

Fetal infection following placental localization induced tissue reactions consisting of multi-organ focal necrosis, vasculitis, and hemorrhage, as well as reticulo-endothelial hyperplasia with epithelioid changes, which probably reflect immune stimulation and thus may not be typical for a specific infection. Histologic changes were detected first 12-27 days after maternal inoculation and became more pronounced with time in third trimester fetuses, where they assumed features of a chronic infection. Although the different fetal lesions are not pathognomonic, since similar lesions may be induced by other infections, the generalized distribution and the pattern of fetal lesion may be helpful in diagnosis.

Immune aspects were not analyzed in this report. Fundamental investigations to gain insight into immune mechanisms are needed to provide methods of prevention and control. The complement fixation test has been the only serologic means to trace chlamydial antibodies in abortion diseases (1, 28). More specific tests with higher sensitivity should now be developed that take advantage of specific chlamydial properties. Chlamydial agents associated with intrauterine infections and abortions in cattle and sheep can be distinguished from other chlamydiae infecting these species. Finally, interdisciplinary investigations might explain and differentiate the syndrome of the so-called epizootic bovine abortion as it occurs in California (4, 20, 22). Chlamydiae reliably inducing placental and fetal infections that lead to abortion, stillbirth, or the birth of weak calves remain the only infectious principle isolated from fetuses of this abortion syndrome.

Summary

Placental and fetal infections of pregnant cows with chlamydial agents leading to abortion or the birth of stillborn or weak live calves represent a distinct etiological disease entity. The worldwide occurrence of this disease corresponds with the distribution of bovine infections with chlamydiae. Abortions may occur as early as the fifth month, but most are seen in the last trimester of gestation. They may occur sporadically, yet the incidence may reach 20% or higher in some herds. This abortion disease is principally diagnosed through isolation of the causative chlamydiae. Since these fastidious obligate intracellular pathogens are readily inactivated in the course of intrauterine infections, this disease is probably underdiagnosed.

Placental chlamydial infections are initiated through intercaruncular endometrial infection that spreads to the overlying chorion. Trophoblastic cells in placental arcades later become infected and necrotic. The infection passes the placental junction to reach the fetus, where it can be detected as early as 6 days after maternal inoculation. Tissue reactions with multiple-organ involvement develop then in the fetus. Focal necrosis and localized inflammatory reactions may be present in liver, spleen, kidneys, central nervous system, adrenal glands, lungs, and intestines. Vasculitis is often associated with lesions in these organs.

Exfoliative cytologic examinations of placental samples increase diagnostic possibilities. The complement-fixation test may be useful to identify antibodies against this infection, but more specific and more sensitive tests need to be developed. Chlamydiae from bovine abortions are indistinguishable from those of ovine abortion, and they differ from chlamydiae causing polyarthritis and certain intestinal chlamydial infections.

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