PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 269–275 (1974)

Placentitis and Abortion in Cattle Inoculated with Chlamydiae Isolated from Aborted Human Placental Tissue[1] (38085)

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Placentitis leading to abortion has been reproduced experimentally in cattle with pure cultures of *Chlamydia psittaci* that originally were isolated from aborted bovine fetuses (1–4). These strains multiply primarily in the cotyledons (5) where they cause severe necrosis. They appear not to multiply extensively or cause significant organ damage elsewhere in the dam. As placentomal infection and necrosis increase, fetal death and abortion follow; or, in milder infections, premature birth of a weak calf may result.

In 1967, Schachter (6) reported the isolation of chlamydiae from 4 of 22 specimens of aborted or curetted placental tissues from 22 women being treated in a San Francisco obstetrical clinic. When the growth characteristics of one of these isolants were compared with those of a chlamydial strain isolated from an aborted bovine fetus (1), both strains were identical in their temperature sensitivity patterns (5). This microbiological similarity stimulated our interest in determining whether the human isolant had abortifacient potential as did the bovine abortion strain. Subsequently, the human abortion and bovine abortion strains were inoculated into pregnant cattle. This report describes the clinical response, gross and microscopic tissue changes, and microbiology of these cattle and compares the data with those previously reported on pregnant cattle infected with nonabortifacient chlamydiae (7).

[1] The authors acknowledge with thanks the technical assistance of Joseph A. Wells and Francis O. Glaser.

Materials and Methods. Chlamydiae. An early chicken embryo (CE) passage culture of strain 24A, one of 4 isolants originally recovered from human placental material (6), was obtained from Dr. J. Schachter, University of California, San Francisco. In a personal communication, Dr. Schachter reported the isolant was recovered from curetted placental tissue of a 38-year-old married woman living in San Francisco. She had a history of 7 pregnancies, the last 3 terminated by spontaneous abortion. Strain 24A was passaged 5 times in chicken embryos at NADL before its use in the present experiments.

Strain 59-795 was isolated in 1959 from an aborted bovine fetus and described as the etiologic agent of epizootic bovine abortion (2) and was obtained from Dr. J. Storz in 1963 as an 8th CE passage culture. It was passaged 3 more times before use.

The NADL-1 strain was isolated in 1969 from the feces of a healthy cow in the NADC stock herd (7). A 3rd CE passage culture was used.

Microbiology. Stock pools of CE-propagated chlamydiae of each strain representing 20–30% suspensions of triturated yolk sacs in beef heart broth were stored at −90°C until used. Inoculums were titrated in chicken embryos at the start of each experiment, and ELD_{50}s/ml were calculated by the method of Reed and Muench (10). Chlamydiae-isolation attempts from tissues, fluids, and feces of infected cattle and fetuses were made according to previously published methods (7).

Experimental animals. Pregnant cows, 2–8 years of age, were obtained from the

Holstein–Friesian breeding herd at the NADC. The stage of gestation at the time of inoculation varied from 105 to 201 days. The source herd was healthy and free of disease as described previously (7); however, most animals in the herd were naturally infected with intestinal chlamydiae (the NADL-1 strain referred to above). Cows selected for inoculation with strains 24A and 59-795 were fed chlortetracyline (2 g/cow/day mixed in a feed additive) for 28 days approximately 6 weeks before inoculation. This treatment depressed shedding of intestinal chlamydiae as demonstrated by repeated negative results of chlamydiae-isolation tests of feces before and during the experimentation.

The 2 pregnant cows used for the NADL-1 infectivity tests were chlamydiae-free animals, raised in isolation, and had negative chlamydial serology and chlamydiae-isolation tests before their use. These cows were infected before the present experimentation and data concerning them were reported elsewhere (7), but some of the data are repeated here in Table I for comparison.

Experimental design. Three pregnant cows were intravenously inoculated with 2 ml of a chlamydial suspension containing approximately 1×10^8 chicken embryo LD_{50} (ELD_{50}) of strain 24A, and 3 cows were inoculated with an equal number of chlamydiae of strain 59-795. An additional cow was inoculated with 4×10^6 ELD_{50} of strain 59–795. One cow in each group was killed 30 days postinoculation (PI) to determine the stage of infection in both cow and fetus. The other cows were kept until they aborted; then they were killed and necropsied. Blood and feces from each cow were collected for chlamydiae-isolation attempts, serologic test and white cell counts before inoculation and at 2–3-day intervals thereafter.

Serology. Serums from cows and fetuses were tested for chlamydial group antibodies by complement fixation (CF) and agar gel precipitin methods. The CF antigen was prepared as described previously (8) and the agar gel precipitin antigen was a sodium deoxycholate extract (9) of purified suspension of strain 59-795.

Histopathology. Tissues of various organs and membranes were collected from aborted fetuses or from euthanatized dams and fetuses at necropsy and fixed in 10% salinized formalin for histopathologic examination. After fixation, tissues were dehydrated in ethanol, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin stain. Impression smears and thin sections of selected fetal organs, placental membranes, and cotyledons were stained with Giemsa and Giménez stains and examined for the presence of chlamydial organisms.

Results. Clinical responses. All cows inoculated with human strain 24A or bovine strain 59-795 developed a hyperthermia (41.1–41.6°C) within 48 hr postinoculation (PI). Rectal temperatures subsided to normal (38.7°C) by the 4th day. Total blood cell counts decreased from an average of 8,600 before inoculation to 4,100 by the 3rd day PI and returned to normal by the 5th day. The clinical course in infected cows was uneventful except for the initial transient hyperthermia after inoculation and a second temperature spike to 40.0°C just before abortion.

Macroscopic lesions. The only significant gross lesions observed at necropsy of the dams were those found in the uterine and placental tissues of cows inoculated with the abortifacient strains. Cows killed 30 or 33 days PI had thick, viscid, yellow-grey exudates in the intercotyledonary zones. The exudate was present in large isolated areas of both pregnant and nonpregnant horns of the uterus. The uterine and chorionic epithelium was necrotic. The condition seemed to be progressively invasive, especially at the basal and arcade zones of the cotyledons. Many of the cotyledons were completely separated from the caruncles. There were localized areas of edema in the chorioallantoic membrane. Animals that aborted had extensive lesions, with broad hemorrhagic areas in the cotyledons. However, in all inoculated cows a few cotyledons appeared to be unaffected.

Aborted fetuses or those taken from the uterus at 30–33 days PI usually were thinner than normal. The extremities of some

fetuses, especially aborted ones, were red and swollen. The livers of most fetuses were discolored, and some were enlarged and coated with fibrin. Ascites and hydrothorax were occasional findings.

Microscopic lesions. The microscopic lesions were basically the same in all animals infected with the placentotropic strains. The extent of lesions appeared to be time related. Multifocal areas of necrosis and ulceration in the intercaruncular endometrial epithelium was a constant finding even in tissues of animals killed at 30–33 days PI. Between the placentomes, there was a copious fibrinopurulent exudate composed of necrotic epithelium and other cellular debris. There were scattered subepithelial, periglandular infiltrations of mononuclear cells with few to moderate numbers of neutrophils. Many small arteries, arterioles, venules, and capillaries adajcent to necrotic areas had extensive to moderate perivascular accumulations of mononuclear cells. The arcade zone of minimally affected placentomes had areas of dissecting necrosis and exudate that divided the chorioallantoic membrane from the placentomal uterine epithelium. Placentomes with more extensive involvement had fetal and maternal membranes that were separated over the entire surface of the placentome. Large focal areas of intercryptic hemorrhage were present over the surface of severely affected placentomes. Though not a constant finding, there were often large areas of edema in the intercotyledonary chorioallantoic membrane.

The microscopic changes in fetal tissues were minimal. Occasionally there was a slight perivasculitis and scattered multifocal areas of mononuclear cells in fetal livers. Some fetuses had hemorrhage and edema in the subcutaneous tissues.

The demonstration of chlamydial agents in this sections and impression smears were difficult and time-consuming. The organisms could be found erratically in placental tissues and in mononuclear inflammatory cells in fetal livers.

Microbiology. Results of chlamydiae-isolation attempts on tissues of inoculated cattle are shown in Table I. Chlamydiae were readily recovered from necrosed cotyledons from all cows inoculated with the 24A or 59-795 strain. Usually only one passage of tissue homogenates in chicken embryos was required because of the large number of infectious organisms present in the placentomes. Chlamydiae were not recovered consistently from any particular fetal tissue. Positive tissues were lung, blood, or liver (Table I). Usually 2 or 3 blind passages in chicken embryos were necessary to confirm these isolations, indicating that the number of infectious chlamydiae in the original tissues was very low.

As further evidence of localization of the infection in the placentome, chlamydiae were not recovered from the liver, spleen, lung, kidney, milk, peritoneal fluid, or joint fluids of any infected cow, nor were chlamydiae recovered from the blood or fecal samples collected before inoculation or from those collected every 2nd or 3rd day up to 30 days PI.

As previously reported (7), no isolations were made from major organs, blood, or feces of pregnant cows inoculated intravenously with the NADL-1 bovine intestinal strain of *C. psittaci.*

Serology. All infected cows had a marked rise in circulating antibody titer as measured by the CF method. The highest titer rise recorded was that for cow 5222 whose circulating antibody titer rose from 1:16 (preinoculation) to 1:2048 when the cow aborted 32 days PI. Preinoculation titers in some of the experimental cows were due to a previous natural intestinal infection with the NADL-1 strain of *C. psittaci.*

Serum from aborted fetuses or those fetuses taken when the cow was killed 30 or 33 days PI negative by the CF test, but were positive by an agar gel precipitin method (Table I).

Discussion. Clinical, histological, and microbiological observations of pregnant cows inoculated with human abortion strain 24A of *C. psittaci* were comparable in every respect with those inoculated with bovine abortion strain 59-795. The results are compatible with those found by other investigators (1–4, 11) who produced experimental bovine abortions with chlamydiae of bovine origin.

TABLE I. Results of Infectivity Tests in Pregnant Cattle of Chlamydial Abortion Strains of Human and Bovine Origin.

Strain/ cow No.	Days gestation at inoculation	Dose iv (ELD_{50})	Clinical event Day PI	Chlamydial isolations at necropsy				Serology		Fetal serum reaction CF/AGP
				Maternal tissues		Fetal tissues		Maternal CF titer		
				Positive	Negative	Positive	Negative	Preinoculation	Postinoculation	
Human 24A										
C5085	140	1×10^8	Abortion 82 days	C (4/5) [a,f]	L,S,M,B[b]	Lu	L,S,K,B,JF	32[c]	512	−/+
C5440	142	1×10^8	Abortion 42 days	—[d]	L,S,B,JF[b]		L,S,Lu[d]	16	256	−/+
C5330	150	1×10^8	Killed 33 days	C (3/3) ,AF,UF	L,S,B,Lu,F	L,S	PX	8	512	−/+
Bovine 59-795										
C5222	201	1×10^8	Abortion 32 days	C (5/5) ,AF,F	L,S,B,Lu	L,S,B,K,Lu,H		16	2048	−/+
C5008	181	1×10^8	Died[e] 16 days	C (4/4) ,AF	L,B,Lu[b]	B	L,S	4	384	−/+

C4928	172	1×10^8	Died[e] 25 days	C (3/3) ,AF	L,B[b]		L,S,Lu,K,B	16	256	−/+
C5774	163	4×10^6	Killed 30 days	C (5/5) ,UF	AF,S,K	L,S,B	Lu,K	0	48	−/+
Bovine NADL-1										
C5761[c]	123	1.4×10^7	Calved at term		L,UF,F		L,S	0	512 at 30 days PI; Neg. at 150 days PI	
C5790	105	1.4×10^7	Killed 30 days		L,S,AF,C		L,S,H	0	256	
None (Control)										
C5410	235	—	Killed		L,S,C		L,Lu	32	32	−/−

[a] C (4/5) means of 5 cotyledons examined, chlamydiae were isolated from 4.

[b] Samples of blood and rectal contents, collected before inoculation and every 3rd or 4th day after inoculation were also negative for isolation of chlamydiae.

[c] Preinoculation titers were due to natural infection with intestinal chlamydiae (NADL-1 strain).

[d] *Strep. uberis* had overgrown many tissues precluding critical examination for chlamydiae.

[e] Cow died from sudden overwhelming *Cl. perfringens* septicemia of unknown origin.

[f] C = cotyledons, L = liver, S = spleen, B = blood, K = kidney, Lu = lung, UF = uterine fluid, AF = amniotic fluid, JF = joint fluid, H = heart, PX = Peritoneal exudate, F = Feces, AGP = agar gel precipitin.

The microbiologic data confirm that both strains cause an infection that localizes in the placentomes with no apparent spread to other organs of the cow and with only incidental spread to the fetus. Thus, both strains must be considered placentotropic. The primary tissue damage is necrosis of the cotyledons with concurrent nutritional deprivation of the fetus as suggested by its smaller size in comparison with uninfected fetuses. Placentomal damage is so extensive when cows are intravenously inoculated with 4×10^6 ELD_{50} or more of abortifacient chlamydiae that abortion seems inevitable. McKercher *et al.* (11) observed that approximately 1×10^6 ELD_{50} of strain 59-795 was the minimal abortion-producing dose for cattle.

The fact that chlamydiae were readily recovered from 24 of 25 cotyledons of cows infected with strains 24A or 59-795 and very inconsistently recovered from fetal tissues indicates that for practical field diagnosis, cotyledons should be the principal tissue taken for chlamydiae-isolation attempts. From the serologic data, it would seem helpful to test fetal serum by the agar gel precipitin method. This test was positive in each aborted fetus or in those killed at 30–33 days PI. In contrast, serums from the fetus of the uninoculated cow or from 20 other normal bovine fetuses collected at an Iowa slaughterhouse were negative by the agar gel precipitin test.

This work confirms the previous work of Kwapien *et al.* (4) that cows naturally infected with intestinal chlamydiae are susceptible to infection and abortion by abortifacient chlamydiae and that the site of primary cellular damage is the placentome.

It is of epizootiologic interest that chlamydiae were not isolated from the blood of the infected cows during the first 30 days PI. The lack of any evidence of chlamydiemia during the acute stages of infection and after abortion seems to suggest that the natural transmission of the infection via sanguivorous arthropods as suggested by various investigators (11) must be very limited.

Summary. The abortifacient potential of a strain of *Chlamydia psittaci* originally isolated by J. Schachter (6) from aborted human placental tissue was tested in pregnant cattle. For comparison, other cattle were inoculated with the prototype bovine abortion strain of Storz *et al.* (1) and with an intestinal strain isolated from cattle feces (7). The human and bovine abortion strains produced severe placentomal necrosis and abortion in intravenously inoculated cows. The clinical signs, gross and microscopic lesions, and serologic reactions caused by either strain were similar. Both strains multiplied and caused their primary damage in the cotyledons but caused little damage elsewhere in the cows. At necropsy, chlamydiae were consistently reisolated from diseased cotyledons and uterine fluids and exudates but not from other maternal organs. Isolations from tissues of aborted fetuses were erratic and inconsistent.

In contrast, as previously reported (7), a bovine intestinal strain of *C. psittaci* failed to cause any significant signs or lesions in intravenously inoculated pregnant cows, and chlamydiae were not reisolated from maternal organs, feces, or fetuses.

These results indicate that the chlamydiae isolated by Schachter from aborted human placental tissues are abortifacient in cattle. They may have abortifacient potential in humans, and therefore they should not necessarily be considered incidental contaminants of the human reproductive tract.

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Received Nov. 19, 1973. P.S.E.B.M., 1974, Vol. 146.