

## Impact of Experimental Genital Mycoplasmosis on Pregnancy Outcome in Sprague-Dawley Rats†

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Specific-pathogen-free (SPF) female Sprague-Dawley rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *Mycoplasma pulmonis* X1048 in 0.1 ml of Frey's broth or with an equal volume of sterile Frey's broth. A minimum of 10 days postinfection, rats were bred to noninfected males. Rats were necropsied at days 11, 14, and 18 of gestation and within 24 h of parturition. Throughout pregnancy, at least 50% of rats remained infected in the lower genital tract. At parturition, the major site of colonization was the respiratory tract ( $P = 0.02$ ). *M. pulmonis* was not isolated from any site of any control rat. Pregnancy outcome was adversely affected by infection with *M. pulmonis*. Infected rats had significantly smaller litter sizes at day 18 of gestation ( $P \leq 0.01$ ) and at term ( $P \leq 0.004$ ). No statistically significant differences among the gestational stages in infected rats were noted for litter size. Total litter weight is a reflection of individual pup weight and of the number of pups born. Therefore, it was obvious that infected rats would have a significantly lower ( $P \leq 0.008$ ) total litter weight than noninfected controls. However, when individual pup weights were considered, infected pups ( $n = 49$ ) also had significantly lower ( $P \leq 0.0001$ ) birth weights than did noninfected controls ( $n = 68$ ). The incidence of an adverse pregnancy outcome at term (stillbirths, macerated fetuses, or resorptions) was higher ( $P \leq 0.01$ ) in infected rats than in noninfected control rats. No stillborn pups or macerated fetuses were observed in any control term rats ( $n = 5$ ). All control rats had live-born pups. Three infected rats had no live-born offspring. Resorptions were more common in infected rats than in control rats ( $P \leq 0.01$ ). The mean number of resorptions per rat was greater in rats which went to term than in rats necropsied during gestation, indicating that the severity of disease was progressive. The rat is frequently the laboratory animal of choice for a wide variety of reproductive studies, and the experimental parameters that are most often measured (litter size, pup weight, and neonatal survival) were all adversely affected by genital mycoplasmosis. Genital mycoplasmosis is important as an animal model for the interaction of infectious agents and the host during pregnancy as well as in its own right as a confounding variable affecting research projects which use the rat as a model to study reproductive function and physiology.

*Mycoplasma pulmonis*, the etiologic agent of murine respiratory mycoplasmosis, (MRM), is a common pathogen of mice and rats. MRM is considered one of the major diseases of laboratory mice and rats (4, 5). In addition to its role in respiratory disease, *M. pulmonis* is responsible for genital infections and infertility. The rat is more often affected with genital mycoplasmosis than the mouse. In conventionally housed female rats, the incidence of natural genital mycoplasmosis has been estimated to be as high as 40% in some colonies (1, 5, 6). The prevalence of gross lesions in these cases is low, with only about 30% of infected females having gross lesions, most commonly oophoritis and salpingitis. In many cases, no gross changes can be appreciated but microscopic examination often reveals mild metritis. As in the respiratory tract, genital infection progresses slowly but eventually will lead to decreased reproductive success.

In conventionally housed rats, the incidence of genital mycoplasmosis can be as high as 40% in some colonies (1, 5, 14); respiratory mycoplasmosis in conventional colonies approaches 100% (14). The incidence of both respiratory and genital mycoplasmosis in barrier-maintained facilities is much lower but is still a significant problem (14). It has been estimated that *M. pulmonis* genital infection may decrease

rat birth rate by 50 to 100%. Obviously this decreased birth rate has a direct impact on studies of reproductive biology using rats and an indirect impact as a result of the less economical production of this animal resource.

Although the lesions in the genital tract of nonpregnant rats and the overall pregnancy outcome of infected rats have been examined, there has not been a comprehensive examination of the effects of infection during pregnancy and at parturition in the rat. The objective of the current study was to examine the effects of *M. pulmonis* on pregnancy development and outcome at selected times within gestation in Sprague-Dawley rats.

### MATERIALS AND METHODS

**Rats.** Specific-pathogen-free Sprague-Dawley male and female rats were purchased from a commercial vendor (Harlan Sprague Dawley, Inc., Indianapolis, Ind.). These rats were monitored and maintained free of the following pathogens: Sendai virus, H-1 virus, rat coronavirus, sialodacryoadenitis virus, reovirus type 3, Kilham rat virus, Hantaan virus, *M. pulmonis*, respiratory and enteric bacterial pathogens, endoparasites, and ectoparasites. The rats were shipped in filter containers to ensure their specific-pathogen-free status.

**Husbandry.** All rats were housed in Microisolator (Lab Products, Inc., Maywood, N.J.) cages to maintain the various infection groups in separate isolation. Rats received

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autoclaved food (Autoclavable Rodent Laboratory Chow 5010; Purina Mills, Richmond, Ind.) and water ad libitum. Hardwood chip bedding and cages also were autoclaved before use. The cages were opened, and rats were handled only under a laminar flow hood with disinfected, gloved hands. When both infected and noninfected rats were to be handled in the same session, noninfected rats were handled first. Rats were kept in a density of four or fewer rats per cage; pregnant rats were caged individually prior to parturition. Cages were changed twice per week.

**Processing of male rats.** Male specific-pathogen-free Sprague-Dawley rats were processed on the day of arrival. Processing included methoxyflurane inhalant anesthesia to facilitate ear notching for individual identification and blood collection. Males were housed with other males when not used for breeding. A male rat was considered infected with *M. pulmonis* after his first exposure to an infected female. Infected males were not used to breed noninfected control female rats and were not housed with any noninfected rat at any time.

**Experimental infection of female Sprague-Dawley rats.** All procedures were performed under a laminar flow hood. Experimentally infected rats were anesthetized on the day of arrival with 0.8 mg of ketamine (Ketaset, Bristol Laboratories, Syracuse, N.Y.) and 0.8 mg of xylazine (Rompun, Haver-Lockhart, Shawnee, Kans.) intraperitoneally to produce a 30- to 40-min period of anesthesia. *M. pulmonis* X1048 was a gift from M. K. Davidson and J. K. Davis, University of Alabama at Birmingham. Females were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* X1048 in 0.1 ml of Frey's broth and were ear notched with a unique number. Uninfected control females were anesthetized with ketamine and xylazine as described above and given an equal volume of sterile Frey's broth intravaginally. These rats also were ear notched for identification. Each group was processed separately. All rats recovered from anesthesia with their pelvises elevated to facilitate establishment of infection in those receiving the *M. pulmonis* inoculum. Once righting reflexes were recovered, two or three rats of the same treatment group were placed into a Microisolator cage with food and water available. No infected female rat was ever housed with a noninfected female. A minimum of five rats per treatment group were necropsied at each time point.

**Breeding and pregnancy detection.** At 5 to 7 days after processing, infected and noninfected control rats to be bred were exposed to soiled bedding from the cages of male rats to synchronize estrous cycles (bedding from infected males was not used in cages of control female rats). Vaginal cells were examined every other day to detect onset of estrus. Females were considered ready for breeding when cornified vaginal epithelial cells were first noted. The goal was to begin breeding at least 10 days after initial processing of the female rats. A harem breeding system was used. Two or three females at approximately late proestrous or early estrous stage of the estrous cycle were housed with one male rat for 3 days, during which the females would complete one entire estrous cycle.

Although monitoring vaginal cytology gave good results for breeding the noninfected rats, vaginitis with resultant high numbers of leukocytes in vaginal swabs from the infected rats made the vaginal slides for these rats difficult to interpret. Therefore, breeding success in the infected rats was very poor when breeding was conducted according to vaginal cytology results. To maximize breeding success, the breeding protocol for infected rats was altered so that

harems of two or three infected females were housed with a male rat continuously for 7 days. Within this period, each female was expected to go through two complete estrous cycles.

Female rats were given two breeding sessions in which to become pregnant before being judged to be infertile. Approximately 11 to 14 days after removal of the male, abdominal palpation was performed on the females twice weekly to detect pregnancy. Rats for the 11-day-pregnant groups often were chosen on the basis of breeding records alone since small litters were difficult to palpate at this stage. By 14 days of gestation, not only were fetuses palpable, but mammary development had begun, making pregnancy diagnosis easier. Decisions to rebreed a female were generally made by day 14 after initial separation from the male. Pairings for repeat breedings were made at random.

**Necropsy.** A minimum of five experimentally infected and five noninfected female rats per time point were necropsied at days 11, 14, and 18 of gestation and within 24 h of parturition. Necropsy procedures were performed as previously described (2). Rats were anesthetized deeply with sodium pentobarbital injected intraperitoneally and were exsanguinated by transection of one femoral artery and vein. A vaginal swab was obtained for culture. The ventrum was swabbed with 70% ethanol. A ventral midline skin incision that extended from the pubis to the rami of the mandibles was made. The abdominal muscles were incised on the linea alba, beginning at the pubis and extending cranially to the xiphoid process. The incision was extended through the costochondral cartilages unilaterally and ended at the thoracic inlet. The ventral cervical musculature was bluntly dissected to reveal the trachea. The trachea was excised from the larynx cranially to the tracheal bifurcation caudally, with care taken to prevent blood contamination of the lumen. The lumen of the excised trachea was washed with 0.5 ml of sterile phosphate-buffered saline (PBS) which was then cultured as described below.

The uterus was exposed from the abdominal cavity. In term dams, the uterus was washed by injecting 0.5 ml of sterile PBS into the uterine lumen with a 1-ml syringe and a 23-gauge needle and aspirating the PBS back into the syringe. Selected uteri with gross lesions from term dams were saved for histopathology. For pregnant preterm rats, the number of fetal implantations that appeared grossly normal and viable for that time of gestation were noted. Resorptions and other abnormalities were noted. For dams which delivered a term litter, the litter size and individual pup weights were determined. The total numbers of implantation sites, including resorptions and retained fetuses, were noted.

Vaginal swabs were placed in 0.9 ml of Frey's broth and serially diluted 10-fold to  $10^{-5}$  in Frey's broth. A Frey's plate was streaked for qualitative isolation. Tracheal and uterine lavage samples (0.1 ml) from each rat also were serially diluted 10-fold to  $10^{-5}$ . Broth cultures were incubated at 37°C in ambient air. Broth tubes were checked daily for a color change, and the reciprocal of the last dilution to show growth was deemed the color-changing units (CCU). In addition to CCU, CFU were also determined for tracheal and uterine lavage samples. For CFU determination, 0.025 ml of the undiluted sample and of each dilution was plated on Frey's agar and incubated at 37°C in 5% CO<sub>2</sub>. Plates were incubated for 5 days before colonies were counted. Cultures were held for 21 days before being discarded. *M. pulmonis* was identified as previously described (1).

Necropsy and quantitative culture procedures for male rats used for breeding were similar to those for female rats.

One of the 11 males used to breed infected females died spontaneously and was not available for necropsy; the remaining 10 male rats were euthanized when they showed evidence of severe clinical disease or when they were no longer needed for breeding purposes. Tracheal cultures were performed as described for female rats. A preputal swab was obtained, and the epididymis and testes were removed aseptically and minced in 0.9 ml of sterile broth. For males with an obvious head tilt ( $n = 5$ ), the middle ear was cultured as follows. The lower jaws were removed and the tympanic bullae were exposed and swabbed with alcohol. The tympanic bullae were punctured by using a needle and syringe, 0.5 ml of sterile PBS was flushed into the ear, and the fluid was aspirated.

**ELISA for IgM and IgG antibodies to *M. pulmonis*.** Specific antibody to *M. pulmonis* was determined by enzyme-linked immunosorbent assay (ELISA) as previously described (1, 3). All sera were plated in duplicate. Horseradish peroxidase-conjugated sheep anti-rat immunoglobulin M (IgM) and anti-rat IgG (The Binding Site, Ltd., Birmingham, England) were used at 1:200 and 1:10,000 dilutions, respectively. Conjugates were diluted in PBS containing 0.05% Tween 20 and 0.02% sodium azide (PBSTA). The plates were incubated with horseradish peroxidase conjugate for 4 h at 37°C and then washed as previously described (1). A commercially available substrate solution was used (ABTS substrate; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) to develop the plates. ABTS solution (0.1 ml) was added to each well. The plates were developed for 20 min (IgM and IgG) at room temperature. At this point, 0.1 ml of corresponding stop solution (Kirkegaard & Perry Laboratories, Inc.) diluted 1:1 with distilled water was added to the wells and the plates were read on an automated ELISA plate reader at 405 nm.

**Statistical analysis.** Data were analyzed by analysis of variance or contingency analysis, where appropriate. A probability of  $\leq 0.05$  was considered significant.

## RESULTS

**Colonization of the dam by *M. pulmonis*.** *M. pulmonis* was not isolated from any site of any control rat. In experimentally infected dams, *M. pulmonis* was isolated from one or more sites in all but four rats (one was necropsied at day 11 of gestation, two at day 14 of gestation, and one at day 18 of gestation). The results of isolation of *M. pulmonis* from different sites at different stages of infection are shown in Fig. 1. On day 11 of pregnancy, four of five rats were colonized in both the vagina and the uterus, indicating that the infection had established in the lower genital tract and ascended to the upper genital tract. Although some rats had become infected in the trachea by day 11 of gestation, the genital tract was more likely to be colonized. Throughout pregnancy, at least 50% of the rats remained infected in the lower genital tract. However, at parturition the major site of colonization was the respiratory tract ( $P = 0.02$ ). The number of *M. pulmonis* organisms isolated from vaginal swabs of rats at term was much lower (a decrease of 2 to 3 log CCU) than at any other time of gestation (Fig. 2). Conversely, greater numbers of *M. pulmonis* organisms were isolated from the trachea at term and at 11 days of gestation than at other times during pregnancy.

**Histopathology.** Uninfected control rat tissues showed no evidence of inflammation of the uterus or placenta. Polymorphonuclear cells were observed within the uterine lumen and uterine glandular crypts, and foci of polymorphonuclear

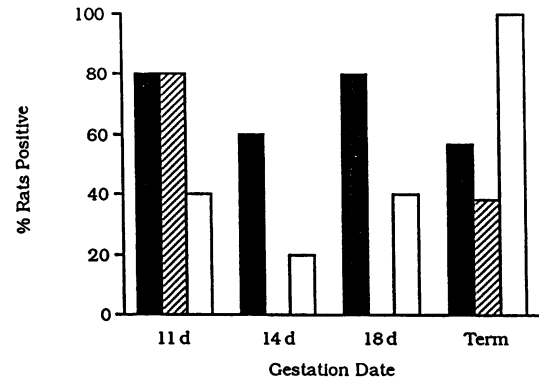


FIG. 1. Percentage of experimentally infected rats from which *M. pulmonis* was isolated from the vagina (closed bars), uterus (hatched bars) or trachea (open bars) at different stages of gestation. All rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* a minimum of 10 days prior to breeding.

cells were invading the maternal layers (decidual and basal layers) of the placenta. One placenta from an infected macerated fetus was severely necrotic with inflammatory cells invading all placental layers. Because the focus of this study was on pregnancy outcome, no evaluations of the male reproductive tract were made.

**ELISA serology.** No antibody to *M. pulmonis* was detected in any control rat or in any experimentally infected rat prior to infection. Infected rats had significantly larger amounts of IgM and IgG at all times tested than did control rats ( $P < 0.001$ ) (data not shown). Both IgM and IgG antibody levels were statistically higher ( $P < 0.001$ ) in serum obtained postinfection (Fig. 3). Although the peak IgM response was observed at day 14 of gestation (24 days postinfection), IgG levels rose steadily during gestation but dropped at parturition. Only two infected rats did not seroconvert in either IgM or IgG. Both rats were culturally negative for *M. pulmonis* at necropsy (days 11 and 18 of gestation).

**Disease in male rats.** No rat used to breed noninfected control females showed clinical signs of disease. After breeding with noninfected control females, males were used to breed infected females. Male rats used to breed infected females were necropsied when they began to show signs of

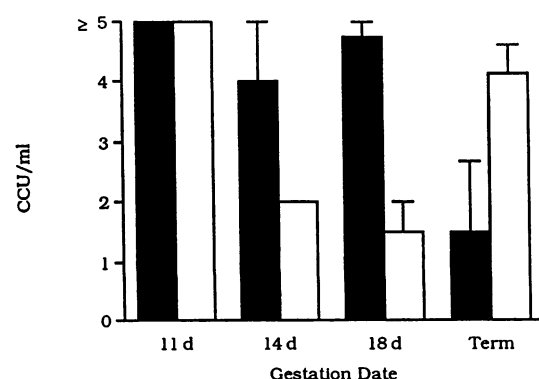


FIG. 2. Numbers of *M. pulmonis* organisms isolated from the vagina (closed bars) and trachea (open bars) of experimentally infected rats at different stages of gestation. All rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* a minimum of 10 days prior to breeding.

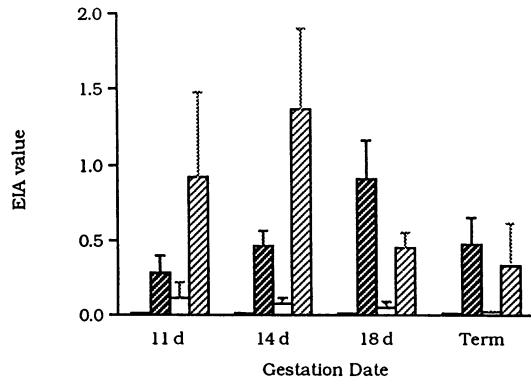


FIG. 3. Serum IgG and IgM antibodies to *M. pulmonis* in experimentally infected rats. All rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* a minimum of 10 days prior to breeding. Prebleed samples were obtained prior to vaginal infection. Postbleed samples were obtained from rats at the time of necropsy. Prebleed IgG and IgM levels (closed and open bars, respectively) and postbleed IgG and IgM levels (darkly and lightly hatched bars, respectively) are shown. EIA, enzyme immunoassay.

disease. Some of the signs noted in these animals included dyspnea, head tilt due to otitis media, anorexia, weight loss, and depression. The mean time from first exposure to the infected females to onset of signs and death was  $52.8 \pm 52.2$  days ( $\pm$  standard deviation), with a range of 178 days (minimum of 23 days and maximum of 200+ days). The mode was 26 days. Five of 11 male rats required euthanasia because of the severity of clinical signs, and one died spontaneously within 35 days of exposure to the infected females. One rat remained clinically healthy, and euthanasia was performed at approximately 200 days postexposure, when the experiment was completed. This rat was only lightly colonized in the respiratory tract (tracheal CCU = 3.0) and middle ear (CCU = 2.0), compared with mean tracheal CCU for all males of  $4.2 \pm 1.6$  and mean middle ear CCU of  $4.4 \pm 1.3$  ( $\pm$  standard deviation), which probably explained his longevity. The site of heaviest growth of *M. pulmonis* from this rat was the testicles, which had a CCU of 4.0 (mean testicular CCU for all males,  $0.6 \pm 1.3$ ). The rat was apparently infertile at the time of euthanasia, with the last fertile breeding having taken place 2 1/2 months earlier. At least one female bred to this rat during the proposed infertile period successfully conceived when bred to another male.

Male rats were colonized in the respiratory tract. *M. pulmonis* was isolated from the tracheas of 9 of 10 rats (90%), with a mean of  $4.2 \pm 1.6$  CCU. Male rats were not as heavily colonized in the reproductive tract as were female rats. *M. pulmonis* was isolated from the prepuce in 2 of 10 rats (20%), with a mean CCU of  $0.5 \pm 1.3$ . *M. pulmonis* was also isolated from the epididymis (mean,  $0.5 \pm 1.1$  CCU) and testes (mean,  $0.6 \pm 1.3$  CCU) of rats with positive preputial cultures. All five rats with head tilt had *M. pulmonis* isolated in high numbers (mean,  $4.4 \pm 1.3$  CCU) from the middle ear.

**Pregnancy outcome.** Pregnancy outcome was adversely affected by infection with *M. pulmonis*. Litter size was defined as the number of pups delivered at term, including stillborn pups, or the number of potentially viable fetuses at day 11, 14, or 18 of gestation. Potentially viable fetuses were those which appeared grossly normal at necropsy. Infected rats had significantly smaller litter sizes at day 18 of gestation ( $P \leq 0.01$ ) and at term ( $P \leq 0.004$ ) (Table 1). No statistically

TABLE 1. Effects of *M. pulmonis* infection on litter size, litter weight, and individual pup weight

Group	Length of gestation	Litter size <sup>a</sup> $\pm$ SD	Litter wt <sup>b</sup> $\pm$ SD	Pup wt <sup>c</sup> $\pm$ SD
Infected	11 days	9.2 $\pm$ 6.0		
	14 days	12.3 $\pm$ 1.4		
	18 days	7.2 $\pm$ 3.4		
	Term	8.4 $\pm$ 3.0	51.6 $\pm$ 11.4	6.3 $\pm$ 1.2
Noninfected (control)	11 days	7.8 $\pm$ 6.5		
	14 days	13.3 $\pm$ 1.5		
	18 days	12.8 $\pm$ 1.5		
	Term	13.8 $\pm$ 1.9	99.0 $\pm$ 6.3	7.2 $\pm$ 0.7

<sup>a</sup> Expressed as the mean number of term pups or number of fetal implantations of normal size and appearance for gestation. Litter size was significantly different between infected and noninfected control rats at day 18 of gestation ( $P \leq 0.01$ ) and at term ( $P \leq 0.004$ ).

<sup>b</sup> Expressed as the mean total term litter weight in grams. Litter weight was significantly different between infected and noninfected control rats ( $P \leq 0.008$ ).

<sup>c</sup> Expressed as the mean of individual pup weights in grams. Individual pup weight was significantly different between infected and noninfected control rats ( $P \leq 0.0001$ ).

significant differences among the gestational stages in infected rats were noted for litter size.

Total litter weight was determined by combining the individual weights of all live-born pups in a litter (Table 1). All pups were weighed within 24 h of parturition. Total litter weight is a reflection of individual pup weight and of the number of pups born. Therefore, it was obvious that infected rats would have a significantly lower total litter weight ( $P \leq 0.008$ ) than would noninfected controls. However, when individual pup weights were considered (Table 1), infected pups ( $n = 49$ ) also had significantly lower birth weights ( $P \leq 0.0001$ ) than noninfected controls ( $n = 68$ ).

The percentages of infected term rats ( $n = 8$ ) with live-born pups, stillborn pups, macerated fetuses, and resorptions are shown in Fig. 4. The incidence of an adverse pregnancy outcome was higher ( $P \leq 0.01$ ) in infected rats than in noninfected control rats. No stillborn pups or macerated fetuses were observed for any control rats ( $n = 5$ ). All control rats had live-born pups. Three infected rats had no

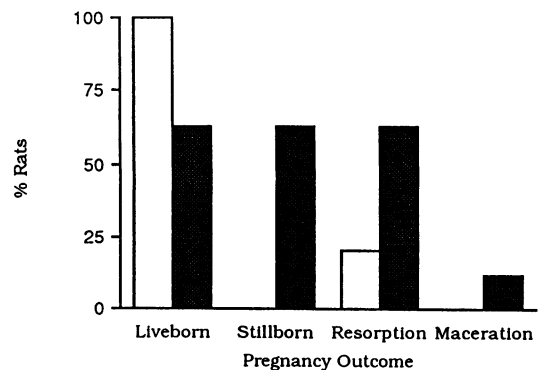


FIG. 4. Pregnancy outcome in experimentally infected and control rats. Rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* a minimum of 10 days prior to breeding or were sham infected with sterile broth. Results are expressed as the percentage of rats with a given outcome. The difference in pregnancy outcome between infected (shaded bars) and control (open bars) rats was statistically significant ( $P \leq 0.01$ ).

live-born offspring (Fig. 4); two of these rats were colonized in the uterus by *M. pulmonis*. All macerated fetuses were obtained from a single rat, which also had three stillborn pups and no live-born pups (Fig. 4). This rat was heavily colonized ( $\geq 10^5$  CFU/ml) in the vagina, uterus, and trachea. A fetus was considered macerated instead of stillborn if it had reached a stage of development similar to that of other full-term live-born pups, was retained in utero, and had undergone postmortem autolysis. The macerated fetuses ( $n = 4$ ) observed in this study usually were covered with purulent material instead of amniotic fluid and were sites of heavy *M. pulmonis* colonization (data not shown). Of the five infected rats with stillborn pups (Fig. 4), four were colonized by *M. pulmonis* in the genital tract and three had no live-born pups.

Resorbed fetuses were those that had obviously implanted in the uterine wall but had become nonviable. They had no recognizable fetal structure remaining. In term rats (Fig. 4), resorptions were noted in five infected rats and one noninfected rat. The noninfected control rat had two resorptions but also had 17 live-born pups. Three infected rats had four or more resorptions, one had one resorption, and one had two resorptions. Resorptions in rats at various gestational stages of pregnancy as well as in those which went to term could be determined. The percentages of rats with one or more resorptions and the number of occurrences of resorption are shown in Fig. 5A and B, respectively. Resorptions were more common in infected rats than in control rats ( $P \leq 0.01$ ). The mean number of resorptions per rat (Fig. 5C) was greatest for rats which went to term, indicating that the severity of disease was progressive. The resorption index was defined as the number of resorptions observed divided by the number of viable fetuses (for gestation age) or the number of live-born pups. The resorption index, shown in Fig. 6, demonstrates the striking loss of viable pups at term in infected animals.

## DISCUSSION

Genital colonization rats in experimentally infected Sprague-Dawley rats in our study are similar to those observed in conventionally housed, naturally infected Sprague-Dawley rats (1, 4, 15). Genital mycoplasmosis is also important in barrier-maintained colonies, in which it may exert even more subtle effects. In a naturally infected, caesarian-derived, barrier-maintained colony of LEW/Tru rats, low levels of infection kept signs of disease masked for months while serology detected antimycoplasmal antibodies in colony rats (6). When signs of disease became evident, not only was MRM seen but decreased fertility was also observed in the breeding females, 80% of whom had *M. pulmonis* isolated from the genital tract.

Infected female rats were exposed to *M. pulmonis* long enough that the microorganism had adequate time to spread to the respiratory tract. At every necropsy time point, at least two infected rats per group had positive tracheal cultures. However, clinical signs of disease were much more limited in the females than in the males. The males used to breed these rats rapidly developed signs of mycoplasmal respiratory disease. Recent evidence for mice supports our finding of increased susceptibility to respiratory disease in males (23). One possible explanation for the rapid colonization of the respiratory tract after a single inoculation of the genital tract is grooming behavior. Intranasal inoculation probably occurred in the males as a result of increased grooming and exploring of the vulva of estrous females, as

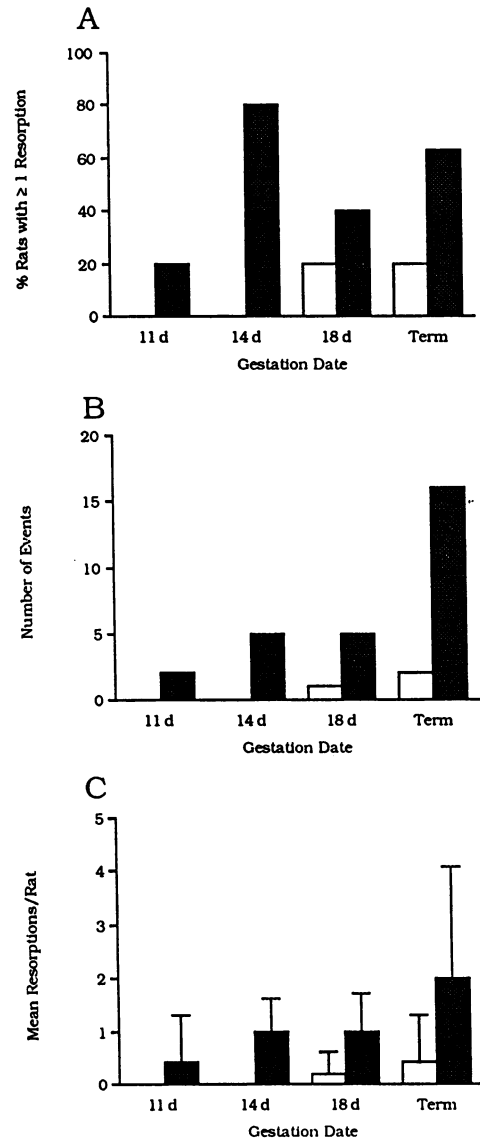


FIG. 5. Fetal resorptions in experimentally infected and control rats at different stages of gestation. Rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* a minimum of 10 days prior to breeding (shaded bars) or sham infected with sterile broth (open bars). Results are expressed as the percentage of rats with one or more resorption (A), the total number of resorptions observed (B), and the mean number of resorptions per rat (C).

well as postcopulatory grooming after breeding genitally infected females (9). Other females may show attention to estrous females similar to that of the male rats and also engage in increased grooming behavior. Once one rat per cage has developed respiratory mycoplasmosis, it becomes a source of aerosolized *M. pulmonis*. As would be expected, the longer the time from initial infection, the more likely the rat is to have respiratory mycoplasmosis (Fig. 5B).

An intriguing observation of the present study is the ability of the rat to clear the genital infection while at the same time the respiratory infection becomes more firmly established. One might expect that an initial infection of the genital tract might be spread to the respiratory tract by orogenital grooming behavior. However, such behavior decreases during

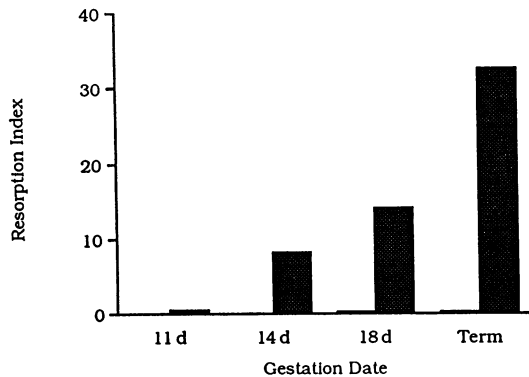


FIG. 6. Resorption index for experimentally infected and control rats at different stages of gestation. Rats were infected by intravaginal inoculation with  $3 \times 10^7$  CFU of *M. pulmonis* a minimum of 10 days prior to breeding or sham infected with sterile broth. Results are expressed as the number of resorptions divided by the number of viable fetuses (11, 14, or 18 days) or live-born pups (term). Data for infected rats (shaded bars) were statistically different ( $P \leq 0.01$ ) from those for control rats (open bars) at all time points except day 11 of gestation.

pregnancy. There is evidence to suggest that colonization of the genital tract is influenced by hormones. In one study, conventional female rats were androgenized at 3 days of age, resulting in a state of constant estrus at sexual maturity (13). All 10 rats showed evidence of oophoritis and metritis, with *M. pulmonis* isolated from the genital tract at necropsy. None of the control rats had these lesions (13). Both the rate of infection in mice and the number of mycoplasmas cultured from mice can be increased by pretreatment with progesterone prior to experimental inoculation intravaginally with *M. pulmonis* and maintenance of increased levels of progesterone postinoculation (8, 21). Even highly passaged *M. pulmonis* organisms (which had greatly decreased hemadsorptive and infective capacities) were able to establish an infection in one progesterone-treated mouse; presumably the few cytoadsorbing organisms left in the passaged inoculum were sufficient to infect when the mouse had high progesterone levels. One hypothesis for this finding is that the decreased mucosal cell turnover occurring under the influence of progesterone on the genital epithelium allows for prolonged cytoadsorption of microorganisms to the genital tract. Adherence of *M. pulmonis* to the mucosa remains the most important factor in initiating infection. Because progesterone remains at high levels throughout pregnancy, the influence of hormones on susceptibility to infection with *M. pulmonis* is likely to play a role in disease severity. In addition, *M. pulmonis* may exhibit a tropism for respiratory tract tissues, perhaps as a function of expression of specific adhesin receptors or other factors. Another intriguing possibility is that the genital tract differs from the respiratory tract in mechanisms by which mycoplasmas are cleared. The decreased occurrence of genital colonization relative to respiratory colonization in naturally infected populations (1, 4, 6) suggests that there occur fundamental differences between the respiratory and genital tracts with respect to mechanisms of colonization or clearance of the microorganisms from the tract or both.

Devastating effects of *M. pulmonis* infection on outcome of pregnancy were seen in experimentally infected rats. Significant fetal losses were observed in the term necropsied dams. Litter size, litter weight, and pup weight also were

decreased. Not surprisingly, those detrimental effects were more evident in the rats that did not clear the genital tract infection. Two of six experimentally infected dams were not genitally colonized by *M. pulmonis* at parturition. Dams which were not colonized by *M. pulmonis* in the genital tract produced litters of 10 and 11 live pups, with one stillbirth total. The dams that were genitally colonized had great fetal losses (only one of four had any live births). We also observed dystocia associated with labor and delivery in the rat which had the macerated fetuses. Areas of striation were prominently embedded on macerated surfaces of the expelled fetuses.

The mechanism(s) by which *M. pulmonis* causes gestational loss are not known. Damage to the uterine endothelium as seen in naturally infected infertile rats (5, 6) may cause fetal death because of the inability of the uterus to adequately nourish the fetus through development. All of the experimentally infected rats had MRM. The stress of this illness on the pregnant rat also may account for some of the fetal loss seen, especially in those rats that were not genitally colonized. In addition, inflammatory products released by the damaged tissue in response to the mycoplasmas may be inhibitory to fetal growth. It has been postulated that one pathogenic mechanism of mycoplasmas is via the release of metabolic products from these bacteria that damage host tissues (16). A toxic environment and stress, directly or indirectly provided by the presence of mycoplasmas, also may be factors in depressed fetal growth. Another intriguing possibility is the interaction of *M. pulmonis* with the host immune system. *M. pulmonis* is a potent immunomodulator and stimulates the production of a number of cytokines (17). Cytokines play a major role in the regulation of pregnancy (10, 11); alteration of normal cytokine levels could result in adverse pregnancy outcome.

The most severe effects were observed in the later stages of pregnancy. One possible explanation for the increased adverse effects in the later stages of pregnancy is that the mycoplasma has simply been present in the genital tract for a longer period of time and has had greater opportunity to exert its effects. Other contributing factors could be decreased cell-mediated immunity and local antibody secretion. The last trimester of pregnancy is associated with decreased cell-mediated immunity and decreased local secretion of immunoglobulins (18, 22). Cell-mediated immunity is important in protection of rats from respiratory mycoplasmosis (7, 12, 20), and a similar role could be involved in protection from the genital disease. Interestingly, the level of specific IgG antibody to *M. pulmonis* decreased at term. In other studies of experimentally infected, nonpregnant rats (19, 20), IgG levels continued to rise well past the time interval in the current study. Our results are consistent with the observed decrease in local secretion of immunoglobulins and suggest that specific humoral antibody to *M. pulmonis* may also be suppressed in the later stages of pregnancy.

The adverse effect of genital mycoplasmosis on pregnancy outcome has been clearly demonstrated. The precise pathogenic mechanism(s) by which *M. pulmonis* causes pregnancy wastage remains to be determined. The disease is important as an animal model for the interaction of infectious agents and the host during pregnancy, as well as in its own right as a confounding variable affecting research projects which use the rat as a model to study reproductive function and physiology.

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