

From the Institute of Medical Microbiology, University of Aarhus, Aarhus, and the State Veterinary Serum Laboratory, Copenhagen, Denmark.

MYCOPLASMOSIS: EXPERIMENTAL AND SPONTANEOUS INFECTIONS OF THE GENITAL TRACT OF BULLS

By

Henning Ernø and Erik Blom

ERNØ, HENNING and ERIK BLOM: *Mycoplasmosis: Experimental and spontaneous infections of the genital tract of bulls.* Acta vet. scand. 1972, 13, 161—174. — A strain of *M. bovis genitalium* was isolated from semen of a bull (K) with chronic seminal vesiculitis. Using this strain a vesiculitis of the same type as found in bull K, characterized by simultaneous acute and chronic lesions, was induced experimentally in 2 bulls by direct inoculation into the vesicular glands. In the acute phase a marked infiltration of eosinophils was found in the interstitial tissues and alveoli whereas in the chronic phase fibrosis, lymphoid and epithelial hyperplasia were seen. Degeneration of vascular walls and connective tissue was common.

On inoculation into the testis of 3 bulls a chronic epididymitis and ampullitis were produced. The histological changes were of the same type as found in the vesicular glands of the 3 bulls with vesiculitis.

By indirect hemagglutination a specific and significant increase in serum antibody could be demonstrated. As the titers were low and the maximum titers were reached early, it will probably not often be possible to make an etiological diagnosis on the basis of serological evidence.

A comparative experiment employing the type strain (PG 11) of *M. bovis genitalium* was performed. A rise in antibody titer was seen, but neither clinical nor histological changes could be demonstrated.

bovine genital mycoplasmosis; seminal vesiculitis; eosinophilia.

Despite the eradication of brucellosis 15 years ago, several cases of seminal vesiculitis in young bulls are found in Denmark every year. Some of the cases are characterized by being abacterial and by the occurrence of pus cells in semen over a prolonged period of time. It is well known that mycoplasmas can be regularly cultured from semen samples, and one must conclude

that in general these microorganisms behave as commensals in the genital tract of bulls. This fact does not exclude the possibility that mycoplasmas at times can cause inflammation of the genital tract, as a result of either predisposing circumstances or occurrence of specific pathogenic strains.

On this basis, the State Veterinary Serum Laboratory initiated a search for mycoplasmas in semen samples containing pus cells. Mycoplasmas were isolated from 5 bulls, and 1 of these animals, K, was purchased for further examination; a series of experimental inoculations with this particular strain, "K", identified as *M. bovigentialium*, were conducted.

The experiments were primarily intended to elucidate the basic question whether strain "K" was able to induce lesions similar to those found in bull K (*Blom & Ernø 1967*). Furthermore, it was of interest to know, if an experimental inoculation was influenced by a preceding spontaneous or induced preputial infection with *M. bovigentialium*. Finally, an experiment was devised to investigate, whether growth factors or preformed toxins which might have been present in the cultures used in the first experiments were necessary for induction of lesions; also serologic responses in bulls inoculated with either the type strain of *M. bovigentialium* (PG 11) or the "K" strain, were compared. The former strain was expected to be non-pathogenic (*Ernø 1972*).

MATERIALS AND METHODS

Bulls

- a. The bull K, Danish Friesian breed, was 1 year old when pus in the semen was first found.
- b. Exps. 1 and 2. Bulls 89 and 90, twins, of Red Danish breed, 19 months old. Mycoplasmas not isolated before inoculation.
- c. Exp. 3. Bull D, Red Danish breed, 2 years old, used for natural service. Before inoculation *M. bovigentialium* was isolated from preputial and semen samples.
- d. Exp. 4. Bull 57, Red Danish breed, 1 year old. Mycoplasmas not isolated before inoculation.
- e. Exps. 5 and 6. Bulls 181 and 182, twins, Danish Friesian breed, 1½ years old. Mycoplasmas not recovered before inoculation.

Isolation and propagation of mycoplasmas

Culture methods and mediums for isolation and propagation of mycoplasmas were similar to those previously reported (*Ernø et al. 1967*).

Clinical examination and examination of semen

All animals were examined clinically before inoculation and at least once a week p.i. Culturing for bacteria and mycoplasmas was performed once a week, as were observations on the appearance of ejaculates, admixture of cells, and quality of sperm. Hemograms were performed in bulls 181 and 182 only.

Examination post mortem

The genital organs were examined macroscopically and samples were taken as required for histologic and microbiological examinations.

Serological examination

The antibody response was determined by the indirect hemagglutination test using fresh, tanned sheep erythrocytes (*Krogsgaard-Jensen* 1971).

EXPERIMENTS AND CASE REPORTS

Bull K*a. Clinical examination and examination of semen*

Clinical examination revealed that the left epididymis and vesicular gland were absent*. The right vesicular gland was of normal size but somewhat firm. Ten ejaculates collected during the last 2 months before slaughtering were of varied quality, often with admixture of pus floccules. All samples had an abnormally high pH (> 7.0) and a low content of dry matter (< 3 %). Sperm morphology was normal. Mycoplasmas were shed intermittently and isolated only twice. The last isolation was made a week before slaughtering. At this time the semen contained 10⁶ colony forming units (c.f.u.)/ml; bacteria could not be demonstrated as a cause of this chronic vesiculitis. The bull was slaughtered after a period of 17 weeks.

b. Examination post mortem

The left vesicular gland and epididymis were practically missing. Left ampule was less developed than usual, being only 6 cm in length. The right vesicular gland was very firm; the cut surface was brownish and dry, and only rather dry drops of secretion could be squeezed from the acini. Histopathological examination revealed lesions in the right vesicular gland only. Areas were found with chronic lesions consisting of fibrosis, stagnation of secretion, atrophy and desquamation of epithelial cells. In other areas the pathologic changes were acute with extensive infiltrations of eosinophilic granulocytes in the interstitia and alveoli, interstitial accumulations of plasma cells, histiocytes and lymphocytes (Fig. 1). Leucocytes with eosinophilic granules had a somewhat varied appearance, a few being large cells with a

* Diagnosis: Segmental aplasia of the Wolffian duct on the left side, a congenital defect described by *Blom & Christensen* (1951).

round pale nucleus; others were similar in size to lymphocytes with a small dark nucleus. However, most cells did have the characteristic appearance of eosinophils, although the cytoplasmic granules were often rather poorly stained and the nucleus often hypersegmented. Intraepithelial occurrence of eosinophils was common (Fig. 2). The alveolar epithelium was at times hyperplastic and folded. Some of the lumina were filled with acidophilic or basophilic amorphous material mixed with cells, occasionally slightly calcified. Focal areas with hyaline degeneration of the connective tissue were seen.

Mycoplasmas were cultured from the preputial cavity but not from the ampullae, testis or vesicular gland. Bacteriological examination was negative. A serum titer of 16 was found in the indirect hemagglutination test (Table 1).

Table 1. Final serum titers of bulls infected with *M. bovis genitalium* as measured by indirect hemagglutination.

Bull	Antigen		Weeks after infection
	strain K	strain PG 11	
K	16	8	> 17
89	16	2	3
90	128	32	8
D	16	8	8
57*	2048	4096	32 + 13
181	64	8	8
182	64	64	8

* Bull 57 was inoculated in the preputial cavity and reinoculated 32 weeks later in testis.

Experiment 1. Bull 89

The animal was inoculated in the vesicular glands through the rectal wall (*Christensen* 1948). A total of 12 ml of a 72-hr. old uncloned culture of strain "K" with 10^5 viable cells per ml was deposited in the cranial, central and caudal parts of both glands. The bull was slaughtered 3 weeks p.i.

a. Clinical examination and examination of semen

On the 3rd day the right vesicular gland was enlarged to about double size while the left one did not appear enlarged until the 14th day. At this time both glands were firm and hard. Gray-brown semen with clots of pus was collected on the 3rd day. Pus admixture continued till slaughtering on the 23rd day. Mycoplasmas were recovered from semen on 4 occasions, but the sample taken the day before slaughtering was negative.

b. Examination post mortem

All genital organs appeared normal except for the vesicular glands. The left one was firm and almost twice as large as normal. The cut surface yielded a creamy yellow secretion. The right vesicular gland was also firm, about 50 % enlarged and had hemorrhagic and pus-filled foci subcapsularly. The cut surface was dry and brownish, with only a sparse secretion present as honey-like drops. Histological examination revealed pathological changes of only the vesicular glands. The lesions were of the same character as those found in the vesicular gland of bull K, but the lymphocytic infiltration was more pronounced, and so were the degenerative and proliferative changes of epithelial cells (Fig. 3). The number of eosinophilic granulocytes and plasma cells was less than seen in bull K. It was typical for this case also that some areas appeared almost normal, whereas the structure of other areas was blurred with inter-alveolar fibrosis, stagnation of secretion, atrophy and rupture resulting in interalveolar connections (Fig. 4). Hyaline degeneration of the walls of blood vessels was seen. Occasionally, epithelial cells with coarsely granular cytoplasm were found, at times even containing inclusion-body-like structures.

Mycoplasmas were recovered from the vesicular glands, the ampules and the prepuce. A titer of 16 was found in serum by the indirect hemagglutination test (Table 1).

Experiment 2. Bull 90

The animal was infected in the left testis, proximally and distally, with a total of 6 ml of the culture used in Exp. 1. The bull was slaughtered after 2 months of observation.

a. Clinical examination and examination of semen

After 1 month the left ampule was firm and slightly enlarged. Two weeks later a hard swelling, the size of a hazel-nut, appeared in the caput epididymidis. On the 22nd day, semen with a heavy admixture of pus cells was collected, and this continued until slaughtering 2 months p.i. Mycoplasmas were recovered after 1 week of observation and all subsequent samples were positive with 1 exception, i.e., the sample taken 3 weeks p.i. The c.f.u. per ml of semen 1 month p.i. was 10^8 . This level was kept for 2 weeks, when the count decreased to 10^3 c.f.u. per ml.

b. Examination post mortem

Macroscopic changes were found in the left testis, epididymis and ampulla. The tunica vaginalis communis was edematous, yellowish, with fibrous adhesions between the 2 layers laterally. The testis itself was strongly distended and edematous. Microscopically a severe lymphoid hyperplasia was found in the mediastinum testis and in the interstitial tissues of the adjacent seminiferous tubules, but apart from this only a few aggregations of round cells were seen in the testis

itself. In the epididymis, lesions of a different type were seen. The caput epididymidis was the site of chronic changes: fibrosis and formation of granulomatous tissue, lymphoid hyperplasia, atrophy and desquamation of epithelial cells liberated to the lumina, at times occluded. Many plasma cells were present, often in a periductular arrangement. Severe lesions occurred in the corpus epididymidis: abscess formation, edema and infiltrations of cells in the capsular and pericapsular tissue. The infiltrations were generally associated with blood vessels, showing endothelial hyperplasia. In the cauda, acute changes were present in the form of necrosis, edema, hemorrhages and cellular infiltrations. The interstitial cells were of the lymphocytic and histiocytic type; whereas the intraductular cells were mainly eosinophils with regular granules or with an edge of eosinophilic cytoplasm. The nuclei were usually round, a few were lobulated. Many blood vessels revealed degeneration of the walls (Fig. 5). In the left ampulla hyperplasia of epithelial cells, fibrosis and focal accumulations of lymphocytes were seen as well as intraepithelial and interstitial infiltration of eosinophils (Fig. 6). Mycoplasmas could be recovered from the left testis, epididymis, ampulla and the preputial cavity. A titer of 128 was found in the serum by the indirect hemagglutination test (Table 1).

Experiment 3. Bull D

This animal was inoculated in the left testis with 5 ml of a 72-hr. old uncloned culture of strain "K" having 10^8 c.f.u. per ml and was slaughtered after a period of observation of 2 months.

a. Clinical examination and examination of semen

As in bull 90 a swelling appeared in the caput epididymidis 6 weeks p.i.; whereas pus cells appeared only 1 week prior to this but in great numbers, as the semen contained 10^8 cells per ml. The shedding of mycoplasmas was intermittent, only every second of 8 samples being positive.

b. Examination post mortem

Macroscopically, an induration of hazel-nut size with central suppuration was found in the left caput epididymidis. Histologically, the lesions in testis, epididymis and ampulla were as described in bull 90. Ductular necrosis and lymphoid hyperplasia are demonstrated in Fig. 7.

Mycoplasmas were isolated from the left ampule, caput epididymidis, testis, prepuce and from the pus found in the caput epididymidis. A titer of 16 was found in serum by the indirect hemagglutination test 8 weeks p.i. (Table 1), whereas no reaction was found 3 weeks p.i.

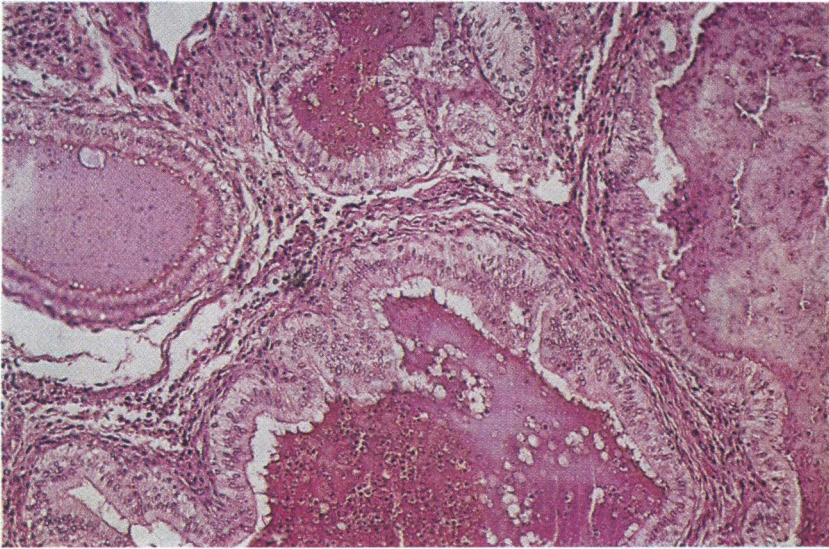


Figure 1. Bull K. Spontaneous seminal vesiculitis. Intra-alveolar accumulations of eosinophils (compare Fig. 2); inter-alveolar infiltrations of mononuclear cells and eosinophils (compare Fig. 2). Epithelial hyperplasia. (HE, 106 \times).

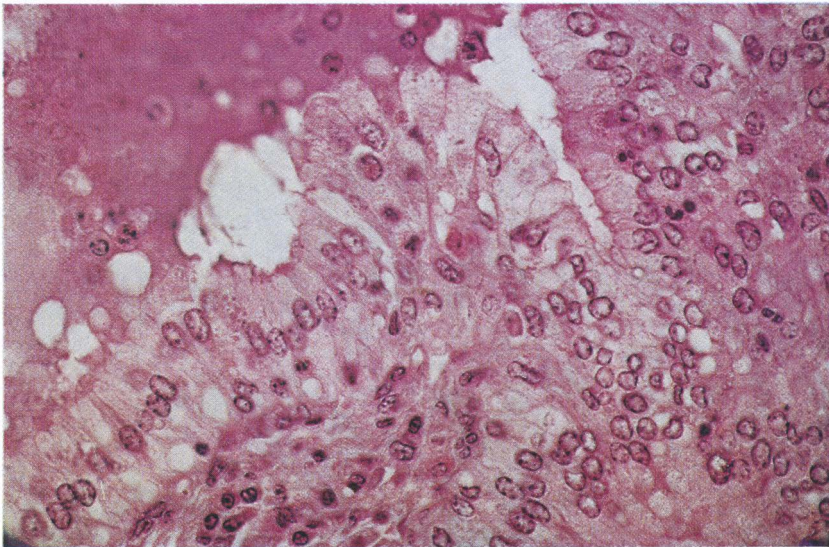


Figure 2. Higher magnification of Figure 1. Inter-alveolar, intraepithelial and intraalveolar eosinophilic granulocytes. Inter-alveolar plasma cells and lymphocytes. (HE, 424 \times).

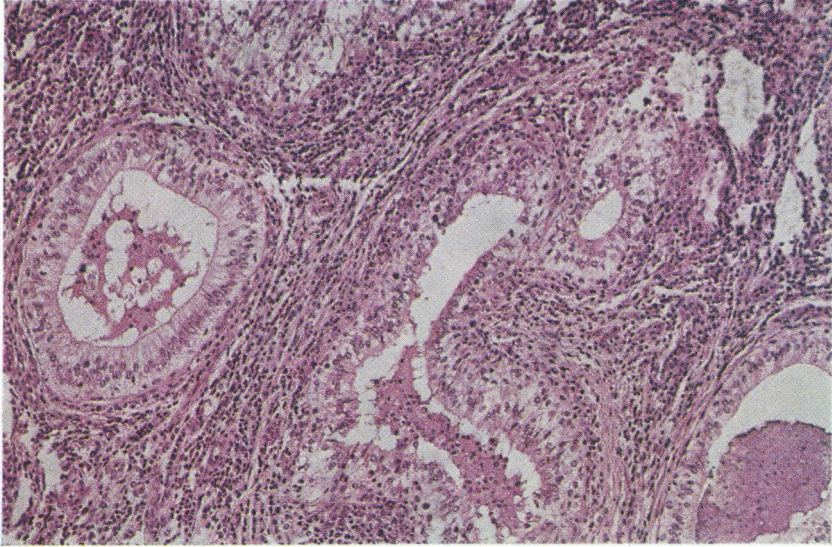


Figure 3. Bull 89. Induced seminal vesiculitis. Intraalveolar accumulations of cells. Degeneration and proliferation of epithelial cells. Severe interstitial infiltration of mononuclear cells. (HE, 106 \times).

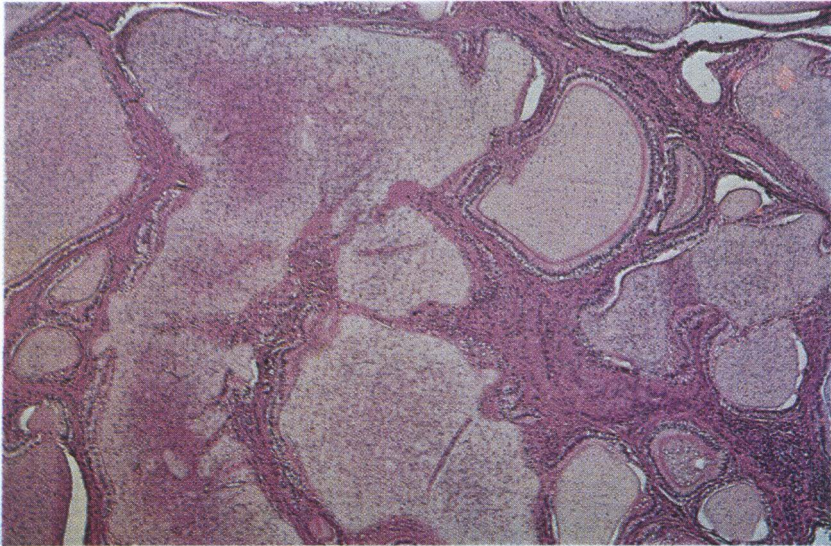


Figure 4. Bull 89. Induced seminal vesiculitis. Interalveolar fibrosis, stagnation of secretion, atrophy of epithelial cells. Interalveolar connections. (HE, 42 \times).

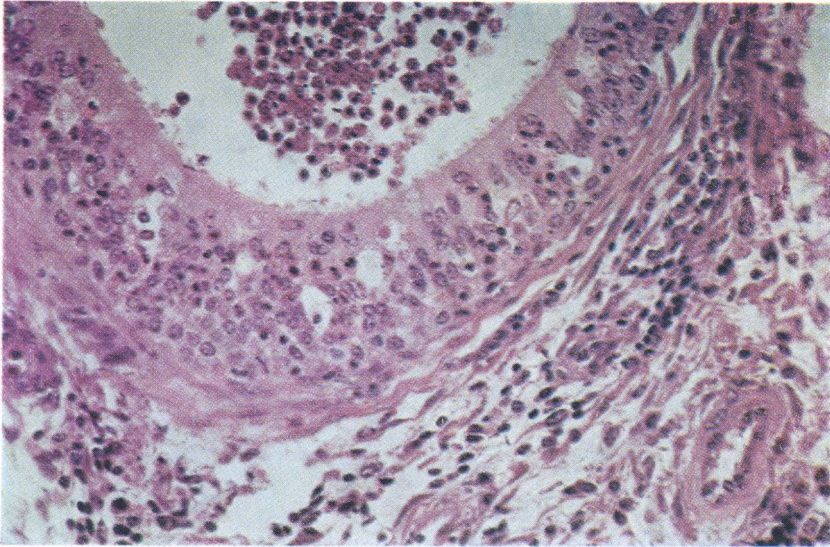


Figure 5. Bull 90. Induced epididymitis. Intraductular cells, mainly eosinophils, occurring also intraepithelially. Interstitial edema, infiltrations mainly of mononuclear cells. Degeneration of blood vessels. (HE, 265 \times).

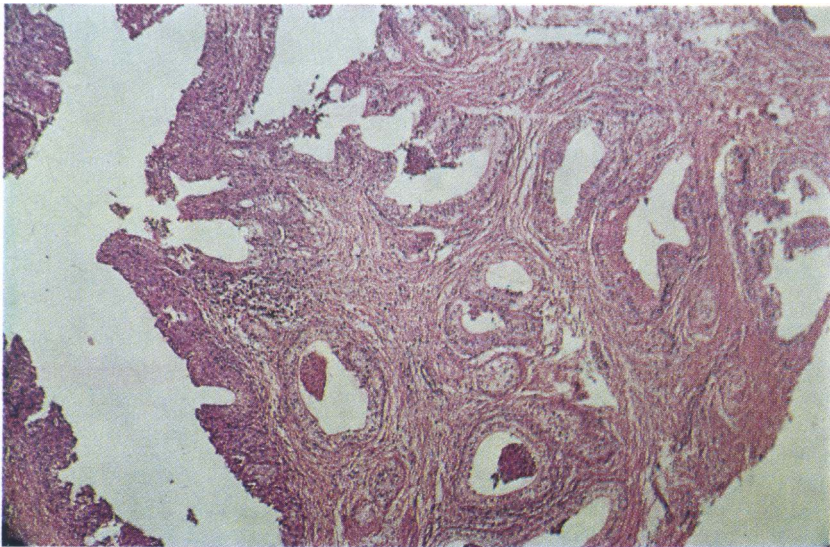


Figure 6. Bull 90. Induced ampullitis. Epithelial hyperplasia, fibrosis, focal accumulations of lymphocytes. (HE, 42 \times).

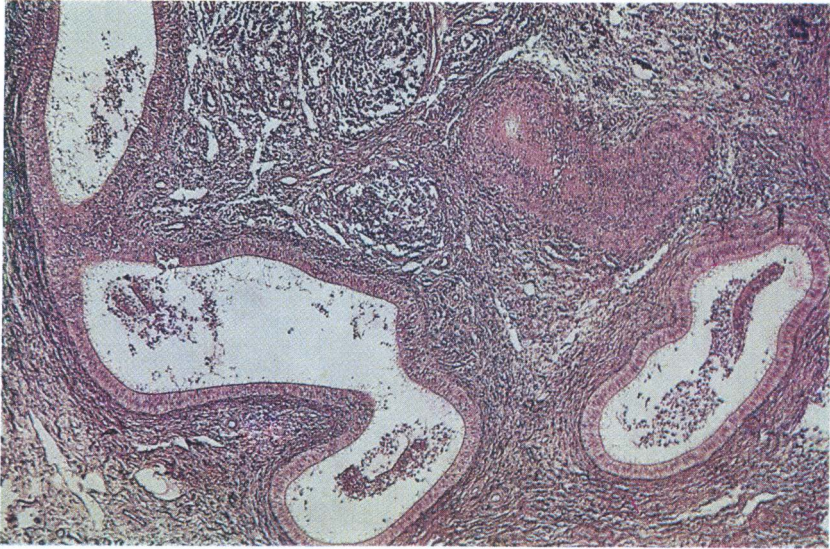


Figure 7. Bull D. Induced epididymitis. Ductular necrosis. Lymphoid hyperplasia. (HE, 42 ×).

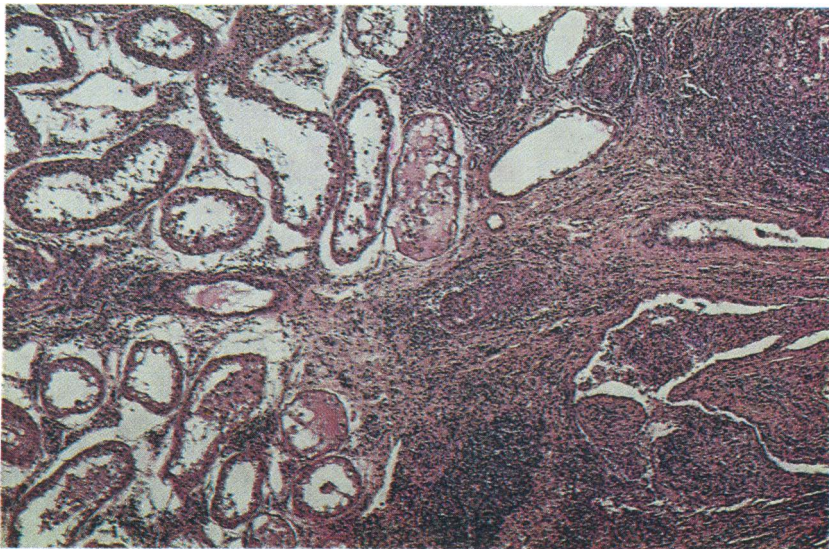


Figure 8. Bull 57. Induced lesions of the rete testis. Lymphoid hyperplasia, fibrosis, necrosis and occlusion of ducts. (HE, 42 ×).

Experiment 4. Bull 57

This animal was inoculated in the preputial cavity with the same culture as used in experiments 2 and 3 and reinoculated 8 months later in the left testis with a total of 10 ml of a 72-hr. old uncloned culture of strain "K" with 10^7 viable cells per ml. The bull was slaughtered 3 months after testicular inoculation.

a. Clinical examination and examination of semen

No clinical symptoms were found until 4 weeks after testicular inoculation when a diffuse swelling in caput epididymidis appeared together with pus cells in the semen. Mycoplasmas were cultured from semen 1 week prior to this and persisted during the remaining period of observation as did the pus cells — though not in great numbers. The epididymal swelling persisted for only 1 month.

b. Examination post mortem

The left testis had a weight of 690 g as compared to 460 g for the right testis. Microscopically, lesions as described in bull 90 were found in the rete testis and epididymis (Fig. 8); although plasma cells were more prominent and eosinophils were not present. The inflammation of the rete testis was predominantly chronic with lymphoid hyperplasia, fibrosis, necrosis, and occlusion of ducts. A chronic, fibrotic and suppurative ampullitis was found. A titer of 2048 was found in serum by the indirect hemagglutination test (Table 1).

Experiments 5 and 6. Bulls 181 and 182

These animals were inoculated in both vesicular glands with washed mycoplasma cells suspended in unenriched broth. Bull 181 was infected with the "K" strain (10^8 viable cells in 12 ml of broth) and bull 182 was inoculated similarly with an equally concentrated suspension of PG 11 (type strain of *M. bovis genitalium*). Both strains were colony cloned at least 3 times. Strain "K" was subcultured 8 times; whereas the passage number of PG 11 is unknown. Both animals were slaughtered after 2 months of observation.

a. Clinical examination and examination of semen

Three days p.i. the left vesicular gland of bull 181 was enlarged, firm and adherent to the surroundings; a few days later it was enlarged $1\frac{1}{2}$ times. Thereafter the swelling gradually subsided; although the gland remained larger and firmer than normal. A little swelling appeared in the anterior part of the right gland 3 days p.i. and disappeared within a week. Mycoplasmas were isolated on the 4th day (10^2 c.f.u. per ml), and the semen remained positive with a maximum (2×10^6 c.f.u. per ml) at 2 weeks p.i. The first ejaculates were dark-brown in color, probably due to hemorrhages following the injections; later a typical admixture of pus floccules appeared.

No clinical symptoms were found in bull 182; the semen remained normal and mycoplasmas were only recovered once, 8 weeks p.i. The hemograms in both bulls were normal throughout the period of observation.

b. Examination post mortem

The left vesicular gland of bull 181 was covered with fibrous tissue (Fig. 9); secretion appeared honey-like and was occasionally coagulated forming small plugs which protruded from the fibrotic cut surface. Secretion from the right gland was brownish and serum-like in the anterior part; microscopically, fibrosis and epithelial proliferations without cellular infiltration were found in this area. The lesions in the left gland were predominantly fibrotic and hyperplastic, but there were areas with intraalveolar and intraepithelial infiltration of eosinophils.

Table 2. Serum titers of bull 181 and bull 182 inoculated with *M. bovigentialium* as measured by indirect hemagglutination.

Bull 181		Bull 182		Weeks after inoculation
Antigen		Antigen		
K	PG 11	PG 11	K	
2	2	8	8	0
32	32	32	16	2
16	16	64	16	4
32	32	64	32	8
64	8	64	64	9

Note: Bull 181 was inoculated with strain "K".
Bull 182 was inoculated with strain PG 11.

Mycoplasmas were recovered from both glands and the preputial cavity. A rise in serum titer (from 2 to 32) was demonstrated in less than 2 weeks. The titer was not significantly changed throughout the remaining period of observation (Table 2).

No lesions were found in bull 182 (Fig. 9), and mycoplasmas were not recovered. However, a significant rise in serum titer was demonstrated (from 8 to 64) during 4 weeks (Table 2).

RESULTS

The experiments clearly prove that *M. bovigentialium* in bulls may be one of the causes of seminal vesiculitis, ampullitis, epididymitis, and inflammation of the rete testis (Table 3). It seems very likely that the chronic vesiculitis in bull K was caused by

Table 3. Summary of clinical and laboratory findings in bulls infected with *M. bovis* genitalium.

Bull	Site of inoculation Experimental period	Clinical diagnoses (Incubation time)	Mycoplasmas in semen Pus cells in semen	Macroscopic diagnoses post mortem	Characteristic histological changes	Mycoplasma positive organs p.m.
K	spontaneously infected 17 weeks or more	chronic, suppurative seminal vesiculitis (not known)	(+) +	chronic, fibrotic seminal vesicu- litis	fibrosis, heavy eosinophilic infiltration, infiltration of mononuclear cells	preputial cavity
89	vesicular gland 3 weeks	acute to chronic suppurative vesiculitis (3 days)	(+) + (3 days p.i.)	chronic, suppurative and fibrotic vesiculitis	infiltration of mononuclear cells, infiltration of eosinophilic granulocytes, fibrosis	vesicular glands, ampules, preputial cavity
181	vesicular gland 8 weeks	acute to chronic suppurative vesiculitis (3 days)	(+) +	chronic, fibrotic seminal vesicu- litis	fibrosis, epithelial hyperplasia, sparse infiltration of eosinophilic granulocytes	vesicular glands, preputial cavity
182	vesicular gland 8 weeks	no abnormal findings	(+) 0	no significant changes	no significant lesions	none
90	left testis 8 weeks	ampullitis (4 weeks) chronic epididymitis (6 weeks)	(+) + (3 weeks p.i.)	edema testis, apostematous epididymitis, chronic ampullitis	fibrosis, infiltration of plasma cells, lymphocytes and eosinophils, necrosis, vascular degeneration	left testis, epididymis, ampule and preputial cavity
D	left testis 8 weeks	chronic epididymitis (6 weeks)	(+) + (5 weeks p.i.)	apostematous epididymitis	as in bull 90	as in bull 90
57	left testis, preputial inoculation 13 weeks	chronic epididymitis (4 weeks)	+ + (4 weeks p.i.)	apostematous epididymitis	many plasma cells, no eosinophilic granulocytes, otherwise as in bull 90	—

Note: Bull K was spontaneously infected with strain "K"; bull 182 was inoculated with strain PG 11; remaining bulls were inoculated with strain "K".

Legend: + : constantly positive
(+) : intermittently positive
0 : negative
— : not examined.

mycoplasmas, since the lesions were of the same type as those induced by experimental infection.

Mycoplasma vesiculitis is characterized by a moderate to marked enlargement of the firmer and sometimes nodular glands. Admixture of cells, even pus floccules are seen in the semen, which may have a brownish color. Sperm morphology appears normal. Mycoplasmas can usually be cultured from semen, but shedding may fluctuate with 1 sample being free of mycoplasmas and the next having 10^6 c.f.u. of mycoplasmas per ml.

The cut surface of the gland is either dry and brownish in color with a sparse secretion presenting itself as honey-like drops occasionally coagulated, forming small protruding plugs, or dry and pale with a thick, yellow, creamy secretion. Histologically, the gland is the site of progressive inflammation with areas of fibrosis, stagnation of secretion, and atrophy of epithelium adjacent to areas with edema and extensive accumulations of eosinophils or to areas with heavy infiltrations of alternate amounts of histiocytes, lymphocytes and plasma cells. Hyaline degene-

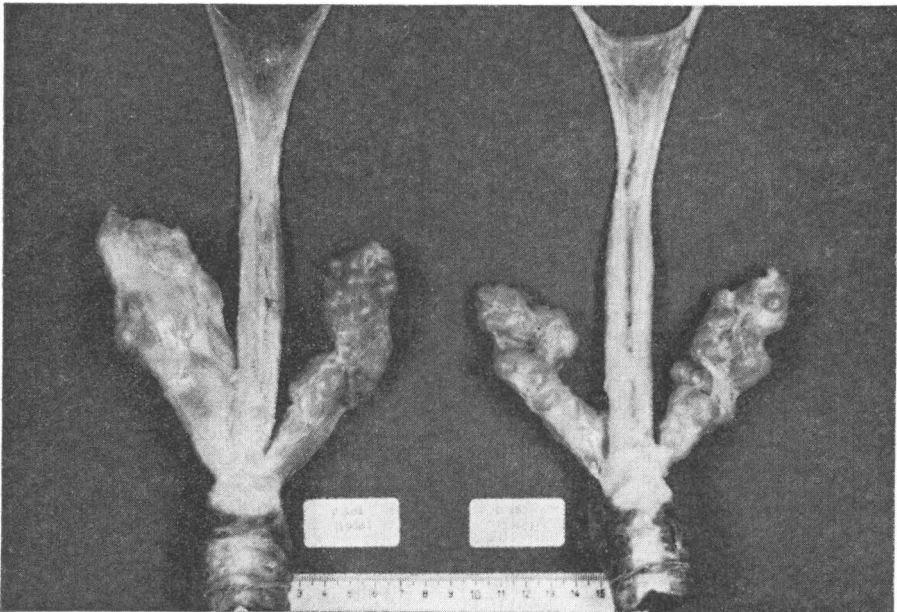


Figure 9. Bulls 181 and 182. Vesicular glands and ampules. Macroscopic changes only in the left vesicular gland of bull 181. Distinct enlargement and capsular fibrosis.

ration of walls of blood vessels and fibrous tissue, as well as epithelial hyper- and metaplasia, are recognized.

Testicular inoculations caused chronic epididymitis and ampullitis in all 3 experiments. In the spermiogenetic tissue only few and insignificant lesions were found, but a severe lymphoid hyperplasia developed in the rete testis. Clinical symptoms were not observed until 4 to 6 weeks p.i. Subsequently variable numbers of pus cells were present in the semen. Mycoplasmas could only be recovered intermittently. Histologic lesions were similar to those present in the vesicular glands, i.e. a progressive acute and chronic inflammation with a more or less pronounced infiltration of eosinophilic cells.

A preceding preputial infection with *M. bovis genitalium* did not alter the course of disease, nor were growth factors in enriched nutrient broth or preformed toxins in cultures of importance for development of lesions. A serologic response was generated, although antibodies were formed only to a low titer. A significant rise in antibodies was seen also following inoculation with PG 11, which did not cause clinical or pathological changes.

DISCUSSION AND CONCLUSIONS

It is evident from the experiments and observations herein reported that it is impossible at this point to make a diagnosis of mycoplasma disease of the genital tract of bulls based on clinical symptoms or routine examinations of semen samples. A species classification is not very helpful as it is still unknown whether some strains are more pathogenic than others within a given species as *M. bovis genitalium*. Neither do serologic tests appear to be of great value in regard to positive diagnoses solely because the maximum titer usually is low and may be reached within less than 10 days after appearance of clinical symptoms. It may be deduced from Table 1, bull 57, that the low titer is not due to a lack of sensitivity of the test as assumed by *Parsonson* (1970), but simply that the actual amount of antibody is small. However, the great sensitivity of the indirect hemagglutination test implies that a repeated negative serologic test is of value in ruling out a given species of mycoplasma as responsible for the said genital disorders.

Our clinical observations agree with the findings of *Al-Aubaidi* (1969) and *Parsonson*. However, it may be deduced from their

experimental and spontaneous cases of mycoplasma vesiculitis that the organisms may have a greater tendency for canalicular and generalized spreading than observed in our material although the significant lesions were still confined to the genital tract, and largely to the seminal vesicles, testis and epididymis. Eosinophilic cells are not mentioned in the work of *Al-Aubaidi*, whereas *Parsonson* observed, on inoculation of *M. bovigentialium* into the seminal vesicles of 13 bulls, that eosinophils were commonly present in the inflammatory cell population in a high percentage. Very reasonably *Parsonson* gives this phenomenon great interest and holds that the persistence of mycoplasmas in the genital tract of the experimental bulls provides a constant source of antigen which could then combine with locally produced antibody to form antigen-antibody complexes that attract eosinophils. We shall confine ourselves to say that the mechanism behind the eosinophilic response remains obscure, not knowing whether antibodies are actually present in the tissues, whether they are locally produced, and if present, homologous or heterologous. What is actually known about the phenomenon is that the eosinophilic response has been reported in mastitis (*Karbe et al.* 1967 and *Mosher et al.* 1968), in endometritis (*Hartmann et al.* 1964) and in salpingitis (*Hirth et al.* 1966) caused by *M. agalactiae* var. *bovis*. It has also been reported in mastitis (*Ernø* 1967), and arthritis (*Ernø* 1969) caused by *M. bovigentialium*. Eosinophils may occur within a few hours in the case of infection of the mammary gland with the former species, which grows rapidly and is uncommon in cattle; whereas the response following infection with *M. bovigentialium* is somewhat slower, indicating a quantitative aspect in the reaction. It was recently reported (*Mosher* 1970) that an immediate eosinophilic skin reaction appeared in cows and pigs intradermally inoculated with purified and concentrated material from *M. agalactiae* var. *bovis* consisting of 73 % carbohydrate and 17 % protein.

Finally, considering the interesting phenomenon of eosinophilic infiltration as described in this paper, the attention is drawn to the work of *Godal & Kåresen* (1967) on histological changes in the thyroid of guinea-pigs following intraperitoneal injection of rabbit anti-guinea-pig thyroglobulin immune serum. Within 24 hrs. an acute eosinophilic response developed, succeeded after 5 days by proliferation of histiocytes and lymphocytes and later by a slight fibrosis. Not only is the cellular

response the same as in the above-mentioned diseases, but even more striking is the resemblance in morphology of the eosinophils which in both cases are somewhat atypical, often having a hypersegmented nucleus with few and poorly stained cytoplasmic granules.

It is interesting to register such similarities in 2 primarily much different diseases. There is every reason to believe that a common pathophysiological mechanism exists, the nature of which is largely an unsolved problem.

REFERENCES

- Al-Aubaidi, J. M.*: Bovine Mycoplasma: Purification, characterization, classification, and pathogenicity. Thesis, Cornell University 1969.
- Blom, E. & N. O. Christensen*: Congenital absence of the epididymis, ductus deferens or glandula vesicularis (Aplasia segmentalis ductus Wolffii) in the bull. Royal Vet. & Agric. College, Yearbook 1951, 1—64.
- Blom, E. & H. Ernø*: Mycoplasmosis: Infections of the genital organs of bulls. Acta vet. scand. 1967, 8, 186—188.
- Christensen, N. O.*: Studies on the agglutinin formation in brucellar infection of the genitals of the bull. Acta path. microbiol. scand. 1948, 25, 202—209.
- Ernø, H.*: Mycoplasmosis: Demonstration of pathogenicity of a Danish strain of mycoplasma. Acta vet. scand. 1967, 8, 184—185.
- Ernø, H.*: Mycoplasmosis: Experimental inoculation of calves with a Danish strain of Mycoplasma bovigenitalium. Acta vet. scand. 1969, 10, 106—107.
- Ernø, H.*: Mycoplasmosis: Serology of infections in the genital tract of bulls. Infect. Immunity 1972, 5, 20—23.
- Ernø, H., W. N. Plastringe & M. E. Tourtellotte*: Mycoplasma: Isolation from prepuce and semen of bulls. Acta vet. scand. 1967, 8, 123—135.
- Godal, T. & R. Kåresen*: Induction of thyroiditis in guinea pigs by serum from rabbits immunized with guinea pig thyroglobulin. Acta path. microbiol. scand. 1967, 69, 332—342.
- Hartmann, H. A., M. E. Tourtellotte, S. W. Nielsen & W. N. Plastringe*: Experimental bovine uterine mycoplasmosis. Res. Vet. Sci. 1964, 5, 303—310.
- Hirth, R. S., S. W. Nielsen & W. N. Plastringe*: Bovine salpingo-oophoritis produced with semen containing a mycoplasma. Path. Vet. 1966, 3, 616—632.
- Karbe, E., S. W. Nielsen & C. F. Helmboldt*: Pathologie der experimentellen Mykoplasmen — Mastitis beim Rind. (Pathology of

- experimental mycoplasma mastitis in the cow). Zbl. Vet. Med., B. 1967, 14, 7—31.
- Krogsgaard-Jensen, A.*: Indirect hemagglutination with mycoplasma antigens: effects of pH on antigen sensitization of tanned fresh and formalinized sheep erythrocytes. Appl. Microbiol. 1971, 22, 756—759.
- Mosher, A. H.*: Patho-biology of a mycoplasmal toxin. Thesis, University of Connecticut 1969. (cit. Vet. Bull. 1970, 40, abstr. no. 2175).
- Mosher, A. H., W. N. Plastridge, M. E. Tourtellotte & C. F. Helmboldt*: Effect of toxin in bovine mycoplasmal mastitis. Amer. J. vet. Res. 1968, 29, 517—522.
- Parsonson, I. M.*: Mycoplasma bovis genitalium: Experimental induction of genital disease in bulls. Thesis, Cornell University 1970.

SAMMENDRAG

Mycoplasmoser: Genitalvejsinfektioner hos tyre.

Fra sædprøver af tyren K med kronisk sædblærebetændelse isoleredes en stamme af *Mycoplasma bovis genitalium*. Med denne induceredes eksperimentelt hos 2 tyre en sædblærebetændelse af samme type som hos tyr K, karakteriseret ved samtidige akutte og kroniske forandringer. I den akutte fase fandtes kraftige ansamlinger af eosinophile granulocytter i det interstitielle bindevæv og alveoler, medens der i den kroniske fase sås fibrose, lymphoid og epithelial hyperplasi. Degeneration af bindevæv og karvægge var almindeligt forekommende.

Ved direkte inokulation i testis hos 3 tyre fremkaldtes kronisk epididymitis og ampullitis. De histologiske forandringer var af samme karakter som fundet hos de 3 tyre med sædblærebetændelse.

Der kunne ved indirekte hæmagglutination påvises en specifik, signifikant antistofstigning, men da titrene var lave og maximum hurtigt nået, vil man næppe ret ofte i praksis kunne stille en ætiologisk diagnose på serologisk grundlag.

Der udførtes et sammenlignende forsøg med typestammen (PG 11) for *M. bovis genitalium*. Efter inokulation i sædblæren fandtes antistofstigning, men der kunne ikke påvises hverken kliniske eller histologiske forandringer.

(Received March 18, 1971).

Reprints may be requested from: E. Blom, State Veterinary Serum Laboratory, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.