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ISOLATION OF MYCOPLASMA FROM BOVINE ABORTED FETI AND WEAK CALVES WITH REFERENCE TO THE HISTOPATHOLOGICAL FINDINGS AND DETECTION OF THE CAUSATIVE SPECIES IN THE FRESH FROZEN TISSUE SECTIONS USING IMMUOFLUORESCENT TEST

(With 2 Tables and 8 Figures)

By

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عزل الميكوبلازما من الأجنة المجهضة والعجول الضعيفة مع إيضاح الظواهر الهستوباثولوجيه وتعيين العترة المسببة فى مقاطع الأنسجة المجمدة بإستخدام إختبار الأميونوفلورسنت

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تـم تجمـيع عـدد ٢٢ جنين مجهض وكذلك ١٢ مشيمة لأبقار مجهضه و٥ عجول ضعيفه وماتـت فى خلال الأسبوع الأول من الولاده و٨ عينات من سائل النفاس للأبقار المجهضه والتى ولدت عجولاً ضعيفه وذلك لفحصها لميكروب الميكوبلازما. عينات من أنسجة المشيمه والكـبد والـرئه والكلـى والقلب وكذلك عينات من محتوى المعده الرابعه للأجنه والعجول الضـعيفه الميته ومن سائل النفاس للأبقار المجهضه أو التى ولدت عجولا ضعيفه تم زرعها وذلـك لعـزل الميكوبلازما. كذلك تم حفظ عينات ممثله لكل عضو فى الفورمالين (١٠%) وذلـك لإسـتخدامها للفحـص الهستوباثولوجى. وأيضاً تم حفظ عينات ممثله لكل عضو فى التجمـيد وذلـك لفحصها بإختبار الأميونوفلورسنت. وقد أظهرت النتائج أن أكثر الأعضاء الـتى تم عزل الميكوبلازما منها كانت الرئه للعجول الضعيفه (٦٠،٠٠%) وللأجنه المجهضه (١٨،١٨ %) يلـيه العـزل من الكبد (٢٠،٠ % و ٩،٠٩ % على التوالى). وفى حين أن مـيكروبات الميكوبلازما لم يتم عزلها من المحتوى المعدى للأجنه الضعيفه، فقد كانت نسبة العـزل من المحتوى المعدى للأجنه المجهضه (٦٤،١٣%). وعند فحص الأنسجه بأختبار الأميونوفلورسـنت فقد تم تعيين عترات الميكوبلازما فى انسجة الرئه للعجول الميته لجميع العيـنات الـتى تم العزل منها(١٠٠ %). وبينما تم تعيين ميكروبات الميكوبلازما بوفس فى أنسـجة كبد(٧٥ %) من الأجنه المجهضه، فإنه قد تم عزل الميكوبلازما بوفس بإستخدام طـريقة الـزرع من (٥٠ %) فقط. وقد تم تعيين ميكروبات الميكوبلازما بإستخدام إختبار الأميونوفلورسنت فى أنسجة كبد (٦٦،٦٧ %) من الأجنه الضعيفه، فى حين أنه قد تم عزل مـيكروبات المـيكوبلازما بإسـتخدام طريقة الزرع من (٣٣،٣٣ %) فقط. وعند الفحص الهستوباثولوجى للرئه والكبد للأجنه المجهضه والعجول الضعيفه كانت الظواهر الباثولوجيه

الأكـثر شـيوعاً هى التهاب رئوى بينى مع التهاب بللورى والتهاب كبدى تتكرزى. الفحص الفلورسـينى لمقاطع الرئه و الكبد للعجول الضعيفه أظهر أن معظم البقع الفلورسينيه تتركز فـى داخـل سيتوبلازم الميكروفاج (الخلايا البلعميه) وبين الخلايا المبطنه للأكياس الهوائيه والشعب الهوائيه وحول الأجزاء المتتركزه من الخلايا الكبديه بالكبد.

SUMMARY

A total of 22 bovine aborted feti as well as 12 placentae, 5 died weak calves and 8 samples of cows' lochia were subjected for *Mycoplasma* isolation. Tissue specimens from placentae and from internal organs of aborted feti and weak calves (liver, lungs, kidneys, spleen and heart) as well as samples from fetal and calf abomasal content and lochia were cultured for *Mycoplasma*. Representative tissue specimens from each organ were subjected for histopathologic examination. Frozen tissue sections from positive cases were subjected for immunofluorescent test. The results revealed that, the most prevalent site of *Mycoplasma* species isolation was from lungs of weak calves and aborted feti (60.0% and 18.18%, respectively) followed by from liver (20.0% and 9.09%, respectively). While *Mycoplasma* organisms couldn't be isolated from abomasal contents of weak calves, the recovery rate from abomasal contents of aborted feti was (13.64%). There was simultaneous isolation of *Mycoplasma* species by conventional cultural method and detection of the same species by immunofluorescent technique (IFT) applied on sections of the frozen tissues of lungs of aborted feti and weak calves (100%). While IFT could detect *Mycoplasma* species in the frozen sections of liver of (75.0%) of the positive aborted feti, the liver of only (50.0%) were colonized *M. bovis*. By IFT *M. bovis* was detected in (66.67%) of weak calves' liver, but by conventional cultural method the organism was identified from only (33.33%) specimens. The most prominent histopathologic lesions that detected on the lung and liver tissues of aborted feti and weak calves were interstitial pneumonia, pleuritis and necrotizing hepatitis. Sections of fresh lung and liver tissues with histopathologic lesions of weak calves, had highly positive fluorescent reaction. *M. bovis* antigen was detected within the cytoplasm of an alveolar macrophage and in cells located in interalveolar septa and in the intercellular bronchial epithelial lining and to the surface of the epithelium of the bronchi, or localized at the perifary of the areas of necrosis, in necrotic exudates and intercelluiary of hepatocytes.

Key words: Mycoplasma, abortion, fetus, fluorescent antibodies, histopathology.

INTRODUCTION

Abortion of farm animals causes serious economic drain on cattle and buffalo industry. The most common infectious causes of abortion in animal are bacterial, viral and protozoal. Isolation of *mycoplasma* species from aborted feti, uterine cotyledons and placental tissues as well as from the genital tract of cattle with history of abortion by many authors (O' Berry *et al.*, 1966, Volintir *et al.*, 1971, Langford, 1975b, Ball *et al.*, 1978, Pfutzner and Schimmel, 1985, Trichard and Jacobsz, 1985 and Byrne *et al.*, 1999), paid attention to a possible association of *Mycoplasma* species with cases of abortion. Studies comparing isolation rates from aborted and non aborted feti have provided evidence that *Mycoplasmas* may play a role in field cases of abortion (Langford, 1975b and Doig and Ruhnke, 1986). While no *Mycoplasma* isolations were made from 33 normal feti and 74 normal placentae, considerably higher isolation rates (8.7%) were reported from cases of abortion. The highest recovery rates were from the fetal membranes (37.5%) followed by the lung, liver and stomach contents (Langford, 1975b). He added that, isolation of *M. bovigenitalium* and *M. bovis* from both the placenta and fetal tissues, suggesting that these organisms were causes of abortions. Ball *et al.* (1978) isolated *Mycoplasma* species from the placentas (23.7%), from aborted fetal material (4.4%), and from the vaginal mucus samples (10.2%) of their dams that taken on the day of abortion. On the contrary, *Mycoplasma* couldn't be isolated from any of non aborting cows and their calves. They concluded that, *Mycoplasma* may play a primary or secondary role in disease of the bovine placenta which results in abortion.

Experimental inoculation of *M. bovis* into the amniotic sac or intra- arterially or intraperitoneally of pregnant heifers resulted in systemic *Mycoplasmosis* with fever and abortion with retention of placenta which led to speculation that hematogenous dissemination might be a prerequisite for colonization of the placenta leading to abortion (Stalheim *et al.*, 1974, Bocklisch *et al.*, 1986 and Doig and Ruhnke, 1986). Byrne *et al.* (1999) concluded that *M. bovis* may be considered as a possible cause of abortion through its ability to cause abortion in experimentally infected cows.

Concerning the weak calf syndrome, Volintir *et al.* (1971) isolated *M. bovigenitalium* from the liver and lungs of 8 aborted feti and from calves born prematurely and died 1-2 days after birth. Page et al. (1972) isolated two *Mycoplasma* strains from an aborted fetus of a cow

and from the placenta of another cow that delivered a typical "weak calf". They denoted that (13%) of calves died after being born weak and (23%) of cows in the affected herd had circulating antibodies against *Mycoplasma* strain. They defined weak calves as the delivery of premature or full-term calves that were too weak to stand and nurse normally. Some calves survived only a few minutes and most "weak calves" died within 1 week.

Isolation and identification of *Mycoplasma* species from tissues of *Mycoplasma* infected animals was not always possible, particularly in chronically affected animals and poor tissue condition. Moreover, gross bacterial contamination could inhibit isolation. So, fluorescent of the frozen tissue sections using homologous antisera may be valuable to visualize the *Mycoplasma* antigens in the affected tissue (Masiga and Stone, 1968, L' Ecuyer and Boulanger, 1970, Stulle *et al.*, 1988). *Mycoplasma hyorhinis* was detected by immunofluorescent (IF) staining with homologous conjugates in frozen cut sections of pneumonic lung tissues from pigs with enzootic pneumonia. They stated that, IF staining was found to be species specific and useful for the early detection and species identification of *Mycoplasma* in tissues. Moreover, it could confirm at necropsy the serological diagnosis of the identified species, L' Ecuyer and Boulanger, (1970). On the other hand, Kirkbride *et al.* (1973) stated that, the efficacy of the fluorescent antibody technique (FAT) would have been higher if tissues were subjected to routine histopathological examination which facilitate detection of the presence of lesions.

So, the aim of the present work was to record the incidence of *Mycoplasma* infection in cases of bovine abortions and weak calves and to detect the most prevalent site of isolation and the identified *Mycoplasma* species. Moreover, to clarify the most prominant histopathological changes in the affected organs of aborted feti and weak calves. Also, the application of fluorescent antibody reaction to detect *Mycoplasma* spp. that responsible for the affection, directly in the fresh frozen tissues of aborted feti and weak calves.

MATERIALS and METHODS

A total of 22 bovine aborted feti at the third trimester (at 6.5 up to 8.5 months of pregnancy) as well as 12 placentas and 3 lochia samples from the aborted cows were received for *Mycoplasma* examination. Moreover, 5 delivered weak calves (full-term calves that were too weak to stand and nurse normally) that died within few hours up to three days

postnatal, as well as 5 samples of their dam's lochia were also received and were subjected for *Mycoplasma* isolation. All the aborted feti as well as the died weak calves were regularely received at the department of reproductive diseases of the institute over two years, from different governorates of Egypt and were culturally negative for brucella and campylobacter.

Postmortem examination: A complete necropsies of aborted feti and weak calves were performed at the laboratory and gross lesions were recorded. Samples from lungs, liver, kidneys, heart, as well as, samples from fetal and calf abomasal contents were collected as aseptically as possible for cultural examination of *Mycoplasma.* Moreover, samples from placentas and lochia were cultured for *Mycoplasma* isolation. Representative samples from each organ were divided into 2 groups, one group stored at room temperature in 10% neutral- buffered formalin for histopathologic examination and the other group of tissues was frozen and sectioned in microtome cryostat for immunofluorescent technique.

Cultural method for primary isolation of *Mycoplasma*: Tissue of each organ (lungs, liver, kidneys and heart, placenta) was flamed, opened with sterile scissors and a small piece (1 mm thick) was taken and inoculated aseptically into heart infusion (HN) broth and shaken with a vortex, three 10-fold dilutions were made, according to the method described by Taoudi *et al.* (1985). A loopful of each sample including abomasal contents and lochia was inoculated aseptically into heart infusion (HN) broth which prepared as described by Freundt (1983) then all the samples were streaked onto the corresponding agar plates. All plates were incubated at 37°C in a humid jar under 10% CO_2 tension and examined for the growth of the typical fried egg appearance.

Genus determination of *Mycoplasma* isolates: Was performed using digitonin sensitivity test as described by Freundt *et al.* (1973).

Biochemical characterization of *Mycoplasma* isolates: was performed according to Erno and Stipkovits (1973) and Barile (1983).

Serological identification: The *Mycoplasma* isolates were identified by well growth inhibition test (WGI) according to Clyde (1964). The isolates were examined against *M. bovis* and *M. bovigenitalium* antisera.

Production of antigen: Antigen was produced according to Senterfit (1983).

Preparation of hyper immune sera: The hyperimmune sera were produced in rabbits according to the method described by Morton and Roberts (1967).

Histopathologic Examination: Tissue specimen from each organ which were fixed in 10% neutral- buffered formalin, routinely processed, and embedded in paraffin in automated tissue processor. Sections were cut at 4-5 µm, stained with hematoxylin and eosin (H&E) and examined by light microscopy (Bancroft and Stevens, 1990).

Immunofluorescent Examination: The frozen part of each organ was sectioned into 8 µ thickness in a microtome cryostat. Fluorescent antibody (FA) technique for the detection of the responsible *Mycoplasma* species was applied to the frozen tissue sections according to (Masiga and Stone, 1968 and Stulle *et al.*, 1988). Briefly, the frozen sections were air dried and fixed in acetone for 20 minutes. Hyperimmune rabbit serum raised against *M. bovis or M. bovigenitalium* was applied to acetone-fixed tissue sections and incubated for 60 minutes at 37°C in a humid incubator. Sections were then washed 3 times with PBS for 10 minutes each, then FITC conjugate (anti-rabbit IgG, whole molecule, antibody developed in goat, Sigma) was applied at dilution of 1/30. The slides were mounted with glycerol phosphate buffer 1:1, covered with cover slide and examined for fluorescence using Carl- Zeiss microscope.

RESULTS

The results presented in table (1) shows that, the recovery rate of *Mycoplasma* species from placentae was (25.0%) relatively higher than from the tissues of the examined aborted feti (18.18%). Moreover, the recovery rate of *Mycoplasma* species from the lochia which collected from aborting cows was relatively high (33.33%). On the other hand, the recovery rate from tissues of weak calves (60.0%) was relatively higher than from lochia of their dams (40.0%).

Concerning the frequency of isolation from the internal organs of aborted feti, the results showed that, the most prevalent site of *Mycoplasma* species isolation was from lungs (18.18%) and the isolated species were (3) *M. bovis* and one *M. bovigenitalium* isolates, followed by from abomasal contents (13.64%) where (2) *M. bovis* and one *M. bovigenitalium* isolates were identified and the least recovery rate was from liver (9.09%) and the isolated species was *M. bovis* (2 isolates). Moreover, the lungs of weak calves were also the most prevalent site of isolation (60.0%) and the identified species was *M. bovis* (3 isolates). While *Mycoplasma* organisms couldn't be isolated from abomasal contents of weak calves, their livers were colonized *M. bovis* in a

percentage of (20%). *Mycoplasma* organisms couldn't be isolated from kidneys, heart and spleen of examined aborted feti and weak calves.

The results in table (2) compared the frequency of *Mycoplasma* species detection by Immunofluorescence technique (IFT) applied to the frozen tissue sections of lung and liver of the positive aborted feti and weak calves and the recovery rate from the same organ by the conventional cultural method. The results showed that, there was simultaneous isolation of *Mycoplasma* species by conventional cultural method and the detection of the same species by immunofluorescent technique applied on sections of the frozen tissues of lungs of aborted feti and weak calves (100%) and the identified species were *M. bovis* (75.0% and 100%, respectively) and one *M. bovigenitalium* isolate was identified from aborted fetus No. 3. While IFT could detect *Mycoplasma* species (2 *M. bovis* and one *M. bovigenitalium*) in the frozen sections of liver of (75.0%) of positive aborted feti, the liver of only 2 out of 4 positive aborted feti (50.0%) were colonized *M. bovis*.

Table 1: *Mycoplasma* recovery rate from examined aborted feti, weak calves, placentae and mother lochia with reference to the isolation site, number of isolates and the identified species.

Examind cases	No. exam.	No. of positive cases (%)	Isolation site Recovery rate (%)	No. of isolates & the identified species
Aborted feti	22	4 (18.18%)	Lung 4 (18.18%)	3 *M. bovis* & 1 *M.bovigenitalium*
			Abomasal cont. 3 (13.64%)	2 *M. bovis* & 1 *M.bovigenitalium*
			Liver 2 (9.09%)	2 *M. bovis*
Placentae of aborted feti	12	3 (25.0%)	Placenta 3 (25.0%)	2 *M. bovis* & 1 *M.bovigenitalium*
Aborted feti's mother lochia	3	1 (33.33%)	Lochia 1 (33.33%)	1 *M. bovis*
Weak calves	5	3 (60.0%)	Lung 3 (60.0%)	3 *M. bovis*
			liver 1 (20.0%)	1 *M. bovis*
Weak calves' mother lochia	5	2 (40.0%)	Lochia 2 (40.0%)	2 *M. bovis*

Table 2: Frequency of *Mycoplasma* species detection by IFT applied to the frozen tissue sections of the positive cases, compared to that recovered by conventional cultural method in relation to the site of isolation.

Positive cases & case's No.	Positive fetal, weak calves tissues & samples					Dam specimens	
	Lungs		Liver		Abomasal contents	Placenta	Lochia
	C.M.	IFT	C.M.	IFT	C.M.	C.M.	C.M.
Aborted fetus 1	+S	+S	+S	+S	+S	N.E.	N.E.
2	+S	+S	-	-	-	+S	+S
3	+G	+G	-	+G	+G	+G	N.E.
4	+S	+S	+S	+S	+S	+S	-
Total	4/4 (100%)	4/4 (100%)	2/4 (50.0%)	3/4 (75.0%)	3/4 (75.0%)	3/3 (100%)	½ (50.0%)
Weak calf 5	+S	+S	-	-	-	N.E	+S
6	+S	+S	-	+S	-	N.E	+S
7	+S	+S	+S	+S	-	N.E	-
Total	3/3 (100%)	3/3 (100%)	1/3 (33.33%)	2/3 (66.67%)	0 (0.0%)	N.E	2/3 (66.67%)

IFT: Immunofluorescence Technique
S : *M. bovis*
N.E.: not examined
- : Negative isolation
G : *M. bovigenitalium*
+ : Positive isolation
C.M. : Cultural method

On the other hand, IFT could detect *M. bovis* in (66.67%) of weak calves' liver, but by conventional cultural method the organism was identified from only (33.33%) specimens.

Regarding to the specimens of the abomasal contents, placenta and lochia, while *Mycoplasma* organisms couldn't be isolated from the abomasal contents of weak calves, abomasal contents of (75.0%) of *Mycoplasma* positive aborted feti were colonized *Mycoplasma* species and the identified species were *M. bovis* (2 isolates) and *M. bovigenitalium* (one isolate). While the placenta of the aborted fetus No. 1 couldn't be examined, the placentae of the three aborted feti were cultured *M. bovis* (2 isolates) and *M. bovigenitalium* (one isolate). Unfortunately, placentae from dams that delivered weak calves couldn't be obtained. Concerning the lochia, *Mycoplasma bovis* was identified from one sample of aborted feti dams' lochia (50.0%), but (66.67%) of the lochias of cows that delivered positive weak calves were colonized *M. bovis*.

Postmortem findings: congestion of all the viscera and the internal organs of the aborted feti and weak calves was the most prominent feature. The lungs of the aborted feti are firm. At necropsy tissues of the aborted fetus No. 1&4 were more oedematous and congested. The examined placentas were soft and friable with patchy thickening and fibrosis. Examination of the weak calf (No. 7) revealed edema of the lungs and liver, the thoracic and peritoneal cavities contained straw-colored fluids.

Histopathological findings: Microscopic examination of sections of lung tissues of the aborted bovine feti revealed the presence of pleuritis and interstitial pneumonia. The broncho-alveolar areas had the most prominent, patchy to diffuse pneumonia. There were lysis and necrosis of the bronchial epithelial lining with desquamated cells with or without many leucocytes mainly alveolar macrophages and scattered neutrophils and lymphocytes in the lumen of terminal airways and that was moderately infiltrated in the interstitial tissue (Fig. 1&2). The alveoli showed thickened alveolar walls and perivascular mild lymphocytic aggregations. The histopathological examination of sections of liver tissue of the aborted feti showed marked diffuse of necrobiotic changes of hepatocytes. Multiple necrotic foci and mild vacuolar degeneration of hepatocytes, in addition to infiltration of lymphocytes and individual neutrophils (Fig. 3&4). Thick oedematous hepatic capsule was observed and that was mildly infiltrated with lymphocytes and few neutrophils.

On the other hand, the histopathological lesions of the weak calves' lungs showed partial stratification in most bronchial and bronchiolar lining epithelium and focal desquamation in few areas. Perialveolar blood capillaries appeared congested, dilated and engorged with blood (Fig. 5) and some of them were hemorrhagic. Although thickening of the alveolar walls by mononuclear cell infiltration and septal cell proliferation was detected in most of the pulmonary tissue, alveolar emphysema was seen in few areas. The pleura showed oedema moderately infiltrated by lymphocytes, plasma cells and histocytes (Fig. 6). The histopathological examination of weak calf liver tissues was similar to those findings in the liver of aborted feti but with severe reaction and was more progressive.

Fluorescent antibody examination:

Sections of fresh lung tissues with histopathologic lesions of aborted feti and weak calves, had highly positive fluorescent reaction with the detection of causative *Mycoplasma* antigen. A uniform fluorescent background outlining the alveolar structure of the lung with

fluorescent highlights scattered throughout the field. Some of these bright fluorescent spots were embedded within the tissue, others adhered to the surface. *M. bovis* antigen was detected within the cytoplasm of an alveolar macrophage and in cells located in interalveolar septa with anti-*M. bovis* hyperimmune sera of sections of weak calf lung (Fig. 7). The fluorescence occurred as individual brightly fluorescent spots or clumps and seemed to be concentrated on or around the cells and cellular debris, or in the intercellular bronchial epithelial lining, sloughed epithelial cells, and free in the luminal spaces or to the surface of the epithelium of the bronchi and to the exudates which filled the smaller passages.

On the other hand, *Mycoplasma* antigen in the fresh liver sections of aborted feti and weak calves appeared as an intensely fluorescent granules which represented individual *Mycoplasma* cells or clumps. fresh liver sections of weak calves showed that, *M.bovis* antigen was specifically localized at the perifary of the areas of necrosis, in necrotic exudates and intercellulary of hepatocytes (Fig. 8).

DISCUSSION

A number of studies have incriminated *Mycoplasma* as possible cause of abortion. The association of *Mycoplasmas* with spontaneous or experimentally induced cattle abortions had been reported by (Volintir *et al.*, 1971, Stalheim *et al.*, 1974, Langford, 1975b, Stalheim and Proctor, 1976, Ball *et al.*, 1978, Ruben, 1980, Pfutzner and Schimmel, 1985, Trichard and Jacobz, 1985, Bocklisch *et al.*, 1986, Doig and Ruhnke, 1986 and Byrne *et al.*, 1999). The results in table (1) showed that, *Mycoplasma* species were recovered from placentas in a higher percentages (25.0%) than from the tissues of examined aborted feti (18.18%). Moreover, the recovery rate from the lochia which were collected from aborting cows was relatively high (33.33%). These results were in agree with that of Ball *et al.* (1978) who suggested that *Mycoplasmas* may play a primary or secondary role in disease of bovine placenta when they isolated the organism in a percentage of (23.7%) from placenta of aborting cows and in a percentage of (10.2%) from vaginal mucus of aborted cows and (4.4%) from aborted foeti. They added that, low recovery rate from aborted feti may be due to inhibitory effects of fetal tissue lysis or the presence of antibody against *Mycoplasma* in the fetal tissues. On the other hand, they stated that *Mycoplasma* don't form part of the microflora of bovine placentas as they couldn't isolate the organism from non aborting placentas. Trichard

and Jacobsz (1985) Isolated *Mycoplasmas* from only (3.3%) of examined aborted feti and (15%) from placentas and the most identified species was *M. bovigenitalium.*

Concerning the frequency of isolation from the internal organs of aborted feti, the results in table (1) showed that, the most prevalent site of *Mycoplasma* species isolation was from lungs (18.18%) followed by from abomasal contents (13.64%) and the least recovery rate was from liver (9.09%). In this concern, Kirkbride *et al.* (1973) stated that *Mycoplasma* species could be isolated from 22 out of 794 cases of bovine abortion from different tissues as fetal tissues (4), stomach contents (2) and from 16 fetal placentas. Langford (1975a) cultured *Mycoplasma* from the stomach contents of (8.7%) of the examined aborted feti and (25%) of the isolates were identified as *M. bovis*. The results were also in agreement with the findings of Ball *et al.* (1978) who stated that the highest recovery rates were from the fetal membranes followed by the lung, liver and stomach contents. Pfutzner and Schimmel (1985) stated that, *M. bovis* was isolated from viable feti and calves of cows with *M. bovis* mastitis. They stated that, *M. bovis* was transmitted both to the fetus and to the new born calf, infected the respiratory system of the calf.

Concerning the weak calves, the results in table (1) showed that, the recovery rate from tissues of weak calves was (60.0%) relatively higher than from lochia of their dams (40.0%). Concerning the frequency of isolation from the internal organs, the lungs of weak calves were also the most prevalent site of isolation (60.0%), and the three isolated strains were identified as *M. bovis*. While *Mycoplasma* organisms couldn't be isolated from abomasal contents of weak calves, their livers were colonized *M. bovis* in a percentage of (20.0%). Stipkovits *et al.* (2001) stated that nearly half of dairy calves were shedding *Mycoplasmas* at the 5th. day of age and the clinical disease causes up to (10%) mortality as a result of severe serofibrinous pneumonia and surviving calves showed very poor weight gain. Ruben (1980) induced systemic *Mycoplasmosis* with fever and placentitis after intra- arterial injection of *M. bovis* to 9 cows at their third trimester of pregnancy. Five cows aborted 26 days PI and three calves born prematurely with sever pneumonia. Hjerpe and Knight (1972) and Stipkovits *et al.* (1993) declared that, in neonatal calves, intrauterine transmission of *M. bovis* is suspected because severe clinical signs are often observed in the first days after birth.

Regarding to the most identified *Mycoplasma* species in the present study, the results in table (1) showed that, the most prevalent isolated species from the aborted feti was *M.bovis* (7 isolates) followed by *M.bovigenitalium* (2 isolates). The isolated species from the placentas were *M.bovis* (2 isolates) and one *M.bovigenitalium* isolate. The only identified *Mycoplasma* species from weak calves tissues was *M.bovis* (4 isolates). *M. bovis* was the only identified species from the aborted feti mother lochia (one isolate) and weak calves dam's lochia (two isolates). In this concern, many authors stated that, Attempts to produce abortion with *Mycoplasma* species other than *M. bovis* have been unsuccessful (Stalheim *et al.*, 1974). Ball *et al.* (1978) stated that *M. bovis* and *M. bovigenitalium* were recovered from both the placenta and fetal tissues, suggesting that these organisms were causes of the abortion. Trichard and Jacobsz (1985) isolated *Mycoplasmas* from aborted feti and placentas of aborting cows, and the most identified species was *M. bovigenitalium*.

The results in table (2) showed that, there was simultaneous isolation of *Mycoplasma* species by conventional cultural method and detection of the same species on sections of the frozen tissues of lungs of aborted feti and weak calves (100%) by immunofluorescent technique (IFT). While IFT could detect *Mycoplasma* species (2 *M.bovis* and one *M.bovigenitalium* isolates) in the frozen sections of liver of (75.0%) of positive aborted feti, *M. bovis* was isolated from the liver of only 2 out of four positive aborted feti (50.0%). On the other hand, IFT could detect *M. bovis* in (66.67%) of weak calves' liver, but by conventional cultural method the organism was identified from only (33.33%) of specimens. Masiga and Stone (1968) and Stulle *et al.* (1988) stated that fluorescent antibody (F.A.) procedure is rapid and specific and have had wide range of application and could be used to study the pathogenesis of *Mycoplasmal* disease. L' Ecuyer and Boulanger (1970) added that, direct Immunofluorescent staining provide a rapid, simple and specific method for the diagnosis of *Mycoplasma* organism in tissue sections, allowing the visualization of the causative agent. Moreover, it could confirm at necropsy the serological diagnosis of the identified species.

In the present study, the reported histopathological lesions of lung tissues of the aborted bovine feti were in agreement with the findings of Murray (1991) and Sakran *et al.* (2001), but with absence of plasma cells. Rodriguez *et al.* (1995) reported similar lesions in the lungs of two naturally aborted caprine fetus from which *M. mycoides subsp. mycoides* was isolated. In a study of calves which had died due to

pneumonia, Buchvarova and Vesselinova (1989) showed that over a third of lungs were cultured *M. bovis* only, while the rest contained a combination of *M. bovis* with other bacterial pathogen. They concluded that alteration in the lungs were chiefly due to *Mycoplasma* infection. Consistent histopathological lesions were found in lung of (6.0%) of aborted feti, which exhibited a generalized mononuclear inflammatory cell infiltration, accompanied by alveolitis, and necrotic placentitis.

In the present study, with the use of immunofluorescent technique (IFT) applied to the lung and liver tissue sections of weak calves, *M.bovis* antigen could be successfully detected. The pattern and localization of antigen by immunofluorescence test in the examined positive lung and liver (Fig. 7 & 8) coincides with those observed by L'Ecuyer and Boulanger (1970) who added that IFT might offer a rapid and relatively simple technique for the detection of *Mycoplasma* organism in tissues even when the specific antigen was in small numbers or associated with many contaminating organisms. In an immuno-histochemical studies of natural and experimental *Mycoplasma bovis* pneumonia in calves, *M. bovis* antigen was mainly detected at the periphery of the areas of coagulative necrosis, in necrotic exudates and in close association with infiltrating macrophages and neutrophils (Rodriguez *et al.*, 1996). Bancroft and Stevens (1990) stated that FAT is the best in diagnosis if fresh frozen tissues is available and morphological details are not paramount.

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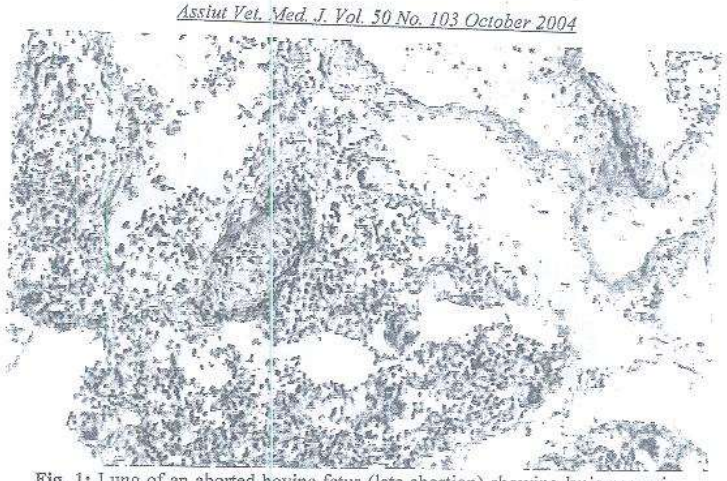

Fig. 1: Lung of an aborted bovine fetus (late abortion) showing lysis necrosis of the bronchial epithelium associated with oedema and inflammatory cells (mainly lymphocytes and few neutrophiles) infiltration in the interstitial tissue. (H&E, X200).

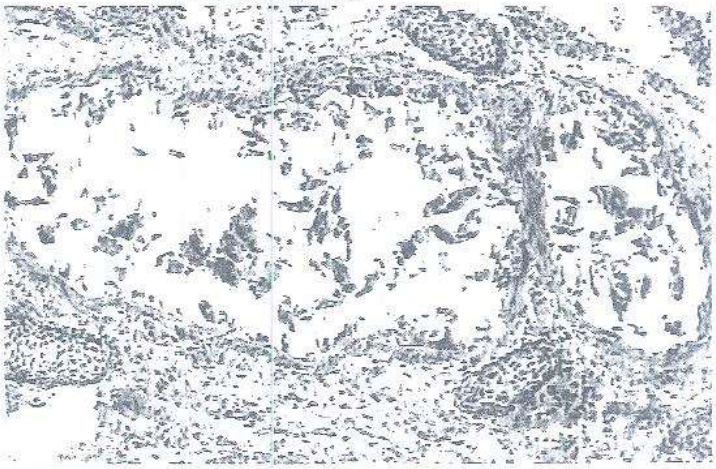

Fig. 2: Lung of an aborted bovine fetus (late abortion) showing lysis and necrosis of the bronchial epithelium associated wth oedema. (H&E, X200).

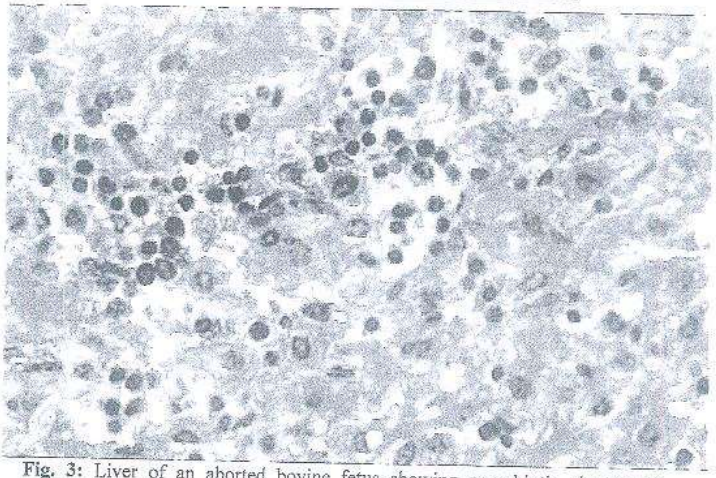

Fig. 3: Liver of an aborted bovine fetus showing necrobiotic changes of hepatocytes with infiltration of lymphocytes and individual neutrophils. (H&E, X400).

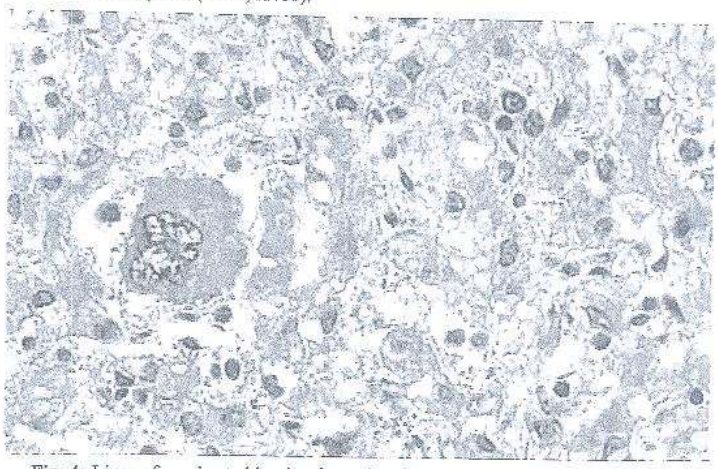

Fig. 4: Liver of an aborted bovine fetus showing lymphocytes cells infiltration with vacuolar degeneration and necrobiotic changes of hepatocytes. (H&E, X400).

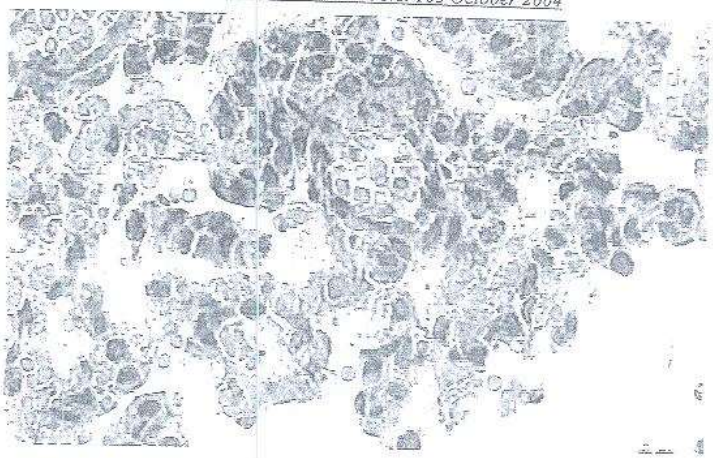

Fig. 5: Lung of weak calf showing severe congestion of alveolar and interstitial blood vessels with extravastion of erthrocytes. (H&E, X400).

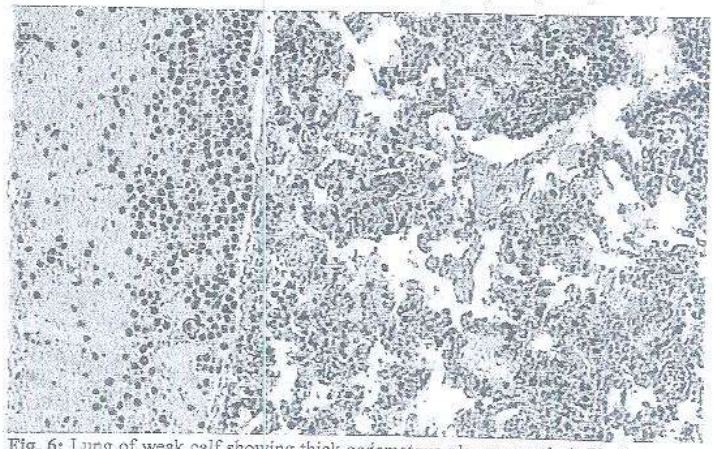

Fig. 6: Lung of weak calf showing thick oedematous pleura severly infiltrated with mononuclear cells, in addition to congestion of alveolar and interstitial blood vessels with lymphocytic infiltration. (H&E, X200).

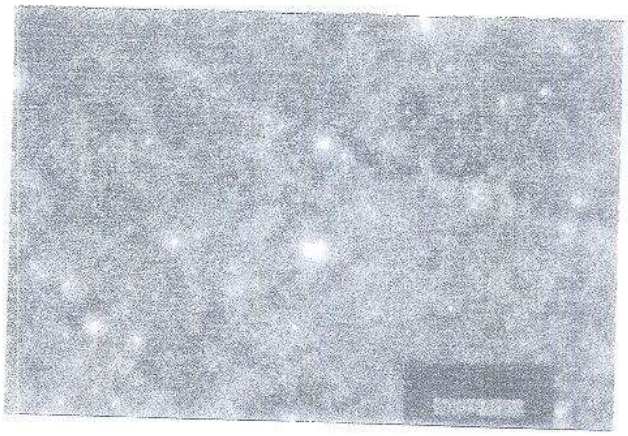

Fig. 7: Frozen section from a pneumonic weak calf lung illustrating the localization of immunofluorescent *M. bovis* antigen (X60).

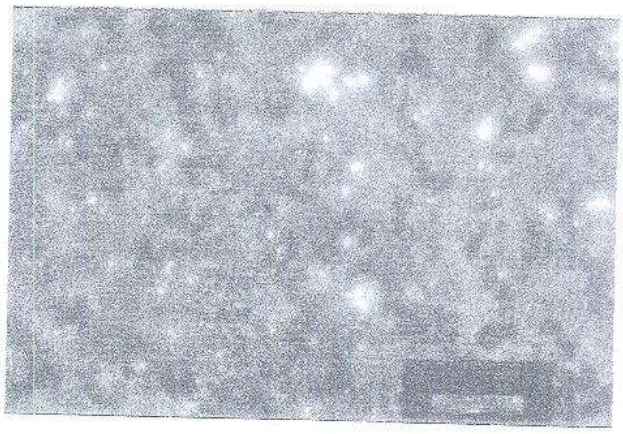

Fig. 8: Hepatic tissue of weak calf stained with direct fluorescent antibody technique showing positive fluorescence for *M. bovis* strains. (X60).