

Mixed Infection of Bovine Viral Diarrhea Virus, Mycoplasma Species and Mannheimia Haemolytica in Calves Showed Chronic Pneumonia with Reference to the Histopathological Findings of the Affected Lungs

Hanaa, A. Ghoneim*, Naglaa, I. Hassan, Hanaa, A. Elhalawany and A.M.Nabih

Animal Reproduction Research institute (ARRI) Giza, Egypt

*hanaeg2002@yahoo.com

Abstract: A total of 100 nasal swabs as well as blood samples were collected from 75 diseased calves suffered from respiratory manifestations and 25 apparently healthy calves of ages ranges from 2-12 month old from three herds. Also 80 clinically pneumonic lung specimens of slaughtered calves were collected from El-warak and El- moneeb abattoir. All were examined to establish the extent of involvement of Bovine Viral Diarrhea (BVDV), *Mycoplasma* species (*M. spp.*) and *Mannheimia. haemolytica* (*M. haemolytica*) in cases of chronic calf pneumonia. On virological studies, AGPT and commercial ELISA kits were rapid and accurate tests for detection of BVDV antigen. BVDV was isolated on MDBK cell line from Buffy coat, nasal swabs collected from diseased calves and lung specimens. The isolated virus was identified by IFAT using reference antisera. Also 100 serum samples collected from diseased and apparently healthy calves were tested by VNT for the detection of neutralizing antibodies against BVDV. Moreover, on bacteriological investigation. *M. haemolytica* were recovered from lung specimens of slaughtered calves as well as nasal swabs of diseased ones and apparently healthy ones. The isolated strains were biotyped as biotype A (56 isolates, 80 %) and biotype T (14 isolates, 20 %). The resistance of the isolates to most antimicrobial agents was high to ceftiofur, nalidixic acid, gentamicin, oxytetracycline, and cephalixin. While they were highly sensitive to norfloxacin, ampicillin and erythromycin. Although, *Mycoplasma species* recovery rate from the examined nasal swabs of pneumonic calves was (46.67%) relatively higher than that recovered from apparently healthy calves (32.00%), the isolation rate from the examined lung tissues reached to (25.0%). The most prevalent isolated species was *M.bovis* followed by *M.dispar*, then glucose positive, arginine negative species. Considering the mixed infection, results showed that, simultaneous isolation of the three pathogens from nasopharyngeal swabs of the examined pneumonic calves was relatively high (12.00%), followed by simultaneous isolation of BVDV & *Mycoplasma sp* as well as *M.haemolytica* & *Mycoplasma sp.* (9.33%). On the other hand, there was simultaneous isolation of both BVDV and *M.haemolytica* from nasopharyngeal swabs of (8.00%) out of the examined pneumonic calves. Examination of 80 clinically pneumonic lung tissues of slaughtered calves that were collected from abattoirs revealed that, a high percentage (17.50%) of examined lung tissues colonized both *Mycoplasma sp.* and *M.haemolytica* together. On the other hand, simultaneous isolation of the three pathogens was detected in (3.75%). However, simultaneous isolation of both BVDV and *Mycoplasma sp. as well as* BVDV and *M.haemolytica* was recorded in (2.50%) of examined lung tissues. Regarding histological studies of lung tissue specimens, there were five types of pneumonia distinguished according to types of necrosis, and cellular infiltrations in relation to microbial isolation, Caseonecrotic bronchopneumonia, 3.75%, Fibrino-necrotizing bronchopneumonia 12.5%, Acute and chronic fibrinosuppurative bronchopneumonia 13.75%. In conclusion *M. bovis* showed two necrotic patterns, where an original focus of coagulative necrosis evolves with time into caseous necrosis ended by fibrosis.

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1. Introduction:

Bovine respiratory tract disease is a multifactorial disease complex and has been one of the most serious problems due to their high mortality and economic losses in calves. This respiratory

disease complex involving several viruses one of these is the bovine viral diarrhea virus. On the other hand, severe respiratory tract disease in cattle is associated with concurrent infections of viruses and bacteria which resulted in suppress the host immune

responses leading to the disease complex. The bacteria that play prominent roles in this diseases complex are *Mannheimia haemolytica* type A, *Mycoplasma* spp as *Mycoplasma bovis*, and *Mycoplasma dispar* (Srikumaran *et al.*, 2007). The occurrence and severity of pneumonia may depend on a series of complex interactions between infectious agents and stress factors as adverse climatic conditions, weaning, transportation, environmental factors and immunological status of the calf (Martin *et al.*, 1982 and Kiorpes *et al.*, 1988). Certain BVDV strains can cause primary respiratory disease and mild respiratory disorder in calves (Archambault *et al.*, 2000 and Baule *et al.*, 2001). The clinical presentation varied according to the age of the affected animal (Jacob *et al.*2010). A synergistic role of BVDV in bovine respiratory disease occur by increasing pathogenicity of both viral and bacterial concomitant infection; this has been attributed to immunosuppressive effects of BVDV on the host (Potgieter, 1997). The immune suppressive effect of acute BVDV infections is due to strong affinity of the virus for immune competent cells which may be destroyed or functionally impaired which enhance the clinical disease of other pathogens and play an important role in multiple infectious diseases (potgieter, 1997).

Mycoplasma bovis, *M. dispar*, *M.bovirhinis* and *Ureaplasma diversum* are four species of mycoplasma that have been established as being of importance as causes of pneumonia in housed calves, based on pathogenicity studies and frequency of association with the disease (Nishimoto and Yamamoto, 2002). *M.bovis* is responsible for at least a quarter to third of calf pneumonia (Nicholas and Ayling (2003). Moreover, Haines *et al.* (2001) reported an increase in cases of antibiotic-resistant pneumonia and fibrinous polyarthritis in which *M. bovis* and BVDV infection were frequently detected. In addition *M.bovis* is considered as an emerging cause of mortality in feedlot cattle and is associated with bronchopneumonia and arthritis (Gagea *et al* , 2006 b).

Mycoplasma dispar is regularly isolated from pneumonic calves but is also found causing mild superficial and asymptomatic infections of the respiratory mucosa (Howard and Taylor 1983). Moreover, it is a proven cause of pneumonia and has been reported in cases of mastitis and was isolated from the lungs and nasal cavities of pneumonic cattle (Muenster *et al.*, 1979). Nishimoto and Yamamoto (2002) reported a case of respiratory mixed infection of *Mycoplasma dispar*, *Manheimia haemolytica* and *pasteurella multocida* of a calf with nine months age. They concluded that, *M. dispar* is presented as the causative agent for pneumonia.

It has been suggested that cattle with primary mycoplasmal infection undergo immunosuppression (Potgieter, 1995), which might predispose to secondary infection with virus or bacteria (Shahriar *et al.*, 2002). Also, Howard *et al.* (1978) concluded that, mixed infections of mycoplasmas and other microorganisms certainly lead to enhanced disease.

Pneumonic pasteurellosis due to *Mannheimia haemolytica* is one of the most important disease complexes causing economic loss in the cattle feedlot industry. It is responsible for the largest cause of mortality in calves farms in Egypt. Shipping fever pneumonia of calves is precipitated by stress-inducing conditions such as shipping, viral infections, inhalation of diesel fumes and overcrowding Frank (1989). Moreover, stress also resulted in nasopharyngeal overgrowth of bacteria, including *M. haemolytica* which results in more bacteria reaching the lungs via inhalation of infected droplets. Cell proliferates under stressful conditions and are erotized in large numbers into lung alveoli, where they cause the disease (Frank, & Briggs. 1992). In addition, respiratory viruses can damage the ciliated epithelium (Andrews & Kennedy, 1997) and compromising the normal defense mechanisms these allowing *M.haemolytica* to colonize the lung. Several mechanisms have been proposed to explain the phenomenon of viral-bacterial synergism in respiratory infection. These include selection of pathogenic form of bacteria, reduced efficiency of lungs in clearing bacteria, depressed phagocytosis or bactericidal potential of alveolar macrophages, depression of ciliary activities and suppression of the immune response (Sharma *et al.*, 1990).

The present study aimed to establish the extent of involvement of BVDV, *Mycoplasma* species and *M.haemolytica* in cases of chronic calf pneumonia. Also, to determine the most prevalent isolated causative agent from the nasopharyngeal specimens of the examined calves in affected herds as well as from the calves' pneumonic lung tissues that collected from abattoir. Furthermore, to throw light on the most pronounced histopathological findings of the affected lung tissues accompanied by each pathogen.

2. Materials and methods

Animal and samples:

A total of 100 nasopharyngeal swabs were collected from calves of ages ranged from 2 up to 12 months old, from which 75 calves were suffered from respiratory manifestations, recumbency, anorexia, abdominal respiration as well as from their closely contact apparently normal calves (25 calves) from three herds at El-kalubia governorate, Miser-

Alexandria road and at the 10th of Ramadan city as shown in table (1). The samples were collected during winter (November, up to April).

Table (1): Number of examined pneumonic as well as closely contact apparently normal calves from the three examined herds

| Herd No. | Pneumonic calves | Closely contact normal calves | Total |
|--------------|------------------|-------------------------------|-------|
| Herd 1 | 35 | 12 | 47 |
| Herd 2 | 22 | 7 | 29 |
| Herd 3 | 18 | 6 | 24 |
| Total | 75 | 25 | 100 |

Two nasopharyngeal swabs were collected aseptically from each examined calf, one sample for *Mycoplasma species* isolation in sterile screw capped tubes containing enriched heart infusion (HN) broth as a transport medium. Another swab was collected on nutrient broth for *Mannheimia haemolytica* cultivation.

More over two blood samples were collected from each examined calf one for serum separation for serodiagnostic tests and the other sample on anticoagulant and the buffy coat were separated for virus isolation and identification. Also, two blood films were freshly prepared from each examined calf for diagnosis of *Mannheimia haemolytica*.

On the other hand, 80 clinically pneumonic lung tissue specimens of slaughtered calves were collected from El-warak and El-moneeb abattoirs, the gross pathological lesions were recorded and representative portions from each pneumonic lung were chosen. Lung tissue specimens were immediately immersed in 10% neutral buffered formalin solution meanwhile the other samples were packed individually in plastic bags and transferred directly to the laboratory in thermos tank with ice packs for virological, mycoplasmal and bacteriological examination. At the laboratory, the naked eye examination of the collected lungs were recorded, and a loopful of deep lung tissues after burning of its surface by very hot spatula were cultured on enriched heart infusion (HN) broth and subsequently on its corresponding agar plates for *Mycoplasma species* isolation, another loopful was inoculated in brain heart infusion agar supplemented with 5% defibrinated sheep blood, blood agar and macconkey agar for *Mannheimia haemolytica* cultivation.

Virological examination:

Agar gel precipitation test (AGPT) according to Hanel (1993). Samples (Buffy coat, nasal swabs and

lung tissues) were tested against standard reference positive hyper immune sera, the agar was used in concentration 1% in PBS and reaction was incubated for 24-48 h at 37°C in CO₂ incubator and examined for the presence of precipitin line.

Institute pourquier ELISA kits:

These kits were used for detection of NSP2-3 of the bovine virus diarrhea/ mucosal disease virus (BVDV).

Inter predation of the results:

Calculate for each sample the S/P ratio (in %) $S/P = \frac{OD \text{ of sample} - OD \text{ of negative control}}{OD \text{ of positive control} - OD \text{ of negative control}} \times 10$ if sample S/P % < or = 25% are considered to be from animals that are not carrier to BVDV. If sample S/P % is between 25 %-30% are considered to be doubtful. If sample S/P % = or > 30% are considered from animals carrier to the BVDV.

3- Cell culture:

Continues cell line of median Darby Bovine kidney cells (MDBK) was used for trials for isolation and propagation of BVDV from (Buffy coat, nasal swabs and lung tissues) which was positive in ELISA test. And also used in virus neutralization test, the cell proved to be free from non-cytopathic strains of BVDV. Monolayer cell line was grown in Eagles MEM supplemented with 10% fetal calf serum. The cell was obtained from virology department Animal Health Research Institute (AHRI). The collected samples after preparation were subjected for virus isolation via propagation on MDBK cell line according to the method described by Clark et al., (1984). Inoculated cells were incubated at 37 °C and were examined daily for 5 days post incubation for three successive blind passages, CPE changes being to appear at the fourth passage.

4- Indirect immuno fluorescent technique (Indirect IFAT):

The (IFAT) was used on the inoculated cell culture with cytopathic effect (CPE) to identify the (cp BVDV), it was used also on inoculated cell culture without CPE to detect (ncp BVDV). The indirect IFAT was carried out according to OIE standers (1992).

5- Enzyme conjugates:

Anti-bovine fluorescence isothiocyanate conjugate was supplied by Sigma immune chemicals used in IFAT.

Reference positive immune sera:

Standard reference positive bovine hyper-immune serum of BVDV was supplied by virology department AHRI.

7- Virus neutralization test:

It was conducted for detection of specific BVDV neutralizing antibodies in cattle serum samples according to OIE (2004).

Isolation and Identification of Mycoplasma species:

The aseptically collected nasal swabs were cultured onto enriched heart infusion (HN) broth and then streaked onto the corresponding agar medium which prepared as described by Freundt (1983) for Mycoplasma species isolation. Naked eye examination of the collected lung tissue specimens were recorded, and a deep part of each lung tissue was cut into small pieces (1mm thick.), inoculated immediately in HN broth medium and shaken with a vortex. Three 10-fold dilutions were made according to the method described by Taoudi et al., (1985) and a loopful of each sample was streaked onto agar plates. All the plates were incubated at 37°C in a humid jar, under 10% CO₂ tension for 72 hours.

Identification of Mycoplasma isolates:

Genus determination: was performed using digitonin sensitivity test according to Freundt et al., (1973).

Biochemical characterization: The isolates were biochemically identified by glucose fermentation test as described by Erno and Stipkovits, (1973) and arginine hydrolysis according to Barile (1983).

Serological identification: It was carried out by growth inhibition (GI) test according to Clyde, (1964). The isolates were serologically examined against rabbit antisera of Mycoplasma bovis, M.bovigenitalium and M. dispar.

Serological test: Serum samples from all examined calves were tested for the presence of M.bovis or M.dispar antibodies by indirect haemagglutination (IHA) test according to Cho et al., (1976). The results were interpreted

Bacterial isolation and identification.

Cultural and biochemical identification:

Nasal swabs which immersed in nutrient broth and a loopful of deep lung tissues after burning of its surface by very hot spatula were cultured on brain heart infusion agar supplemented with 5% defibrinated sheep blood, blood agar and Macconkey agar. Plates were incubated at 37°C for 24 hours (Kodjo, et al. 1999). Bacterial identification was assessed by:

The observation of the colonial morphology

Gram staining and biochemical identification includes oxidase, catalase, urease tests, triple sugar iron agar, motility tests and indol tests (Atlas, 1997; Baily and Scotts, 1998 and Topy and Wilson, 1998).

Blood films Staining:

The freshly prepared blood films from examined calves were stained with Leishman stain and examined under oil immersion lens for detection of Gram-ve biopolar bacilli.

Biotyping of isolated M.haemolytica:

Biotyping of isolated M.haemolytica was applied depending on L-arabinose, trehalose, D-xylose, lactose and salicine fermentation tests (Biberstein et al., 1990).

Antimicrobial susceptibility test (Sensitivity test):

The susceptibilities of isolates to antimicrobial agents were determined by using the disk diffusion method according to the NCCLS Guidelines (2002). The antimicrobial disk used are Ampicillin, Amikacin, Ceftiofur, Cephaloridine, Cephalexin, Erythromycin, Norfloxacin, Oxytetracyclin, Pencillin G, Streptomycin and Nalidixic acid.

Pathological examination:

Pneumonic lung specimens were taken immediately from the slaughtered calves and immersed in 10 % formalin. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin. The embedded samples were sectioned at 3–5 µm thickness, stained with Haematoxylin-Eosin stain and, when necessary, with special stain (Giemsa stain and Periodic acid schiff (PAS) (Bancroft and Marilyn, 2002). The slides were examined using light microscope.

3. Results and Discussion:

Virological results and discussion:

As shown in table (2) the Buffy coat of pneumonic calves showed higher percentage of viral detection by ELISA test (92.00 %) than by AGPT (36.00 %) . Also, detection of BVDV from nasal swabs by ELISA test (76.00 %) was higher than AGPT (25.33 %). Although, BVDV could not be detected by AGPT either in the buffy coat and nasal swabs of apparently healthy calves, the virus was detected in small numbers by ELISA test in buffy coat and nasal swabs (4.00 % and 12.00 % respectively). While BVDV was detected in lung tissues in higher percentage by ELISA test (25.00 %) than by AGPT (6.25 %).

In this study AGPT was used as a simple and rapid method for BVDV or antigen detection in collected samples (nasal swabs and lungs) which reported by (Hanel, 1993; Hosny et al., 1996 and Nahed et al., 2004). Results of AGPT are low if compared with other tests due to the low sensitivity of the test or type of sample collection. Commercial Pourquier ELISA kit (antigen capture ELISA) gave rapid and accurate detection of BVDV antigen in the same original samples.

An antigen capture ELISA was developed and proved its specificity, sensitivity and accuracy (Gottschalk et al., 1992). Also, (Donis, 1995 reported that antigen detection ELISA for BVDV was usefully mainly in confirming enteric, respiratory and reproductive diseases. Many authors described the

importance of ELISA in testing program of animals BVDV (Sandvik et al., 1997 and Ferrari et al., 1999).

ELISA test was improved by the use of monoclonal antibodies which were specific to BVD viral proteins and we could detect most if not all BVDV strains (Sandvik, 1999). In our study we use kits with specific monoclonal antibodies directed to NSP2-3 (NSP-3 associated with lytic activity of the cytopathic strain) which increased the application of ELISA for detection of BVDV strains and offered sensitivity equivalent or higher than virus isolation, this also recorded by (Ferrari et al. 1999; Cavirani et al., 2000 and OIE2000). Also, Aymen, (2002) proved that commercial ELISA kits are valuable in antigen detection.

Table (2): Comparison AGPT and ELISA test for detection of BVD antigen in the diseased, apparently healthy and slaughtered calves.

| State of animal | Sample tested | | +ve by AGPT (%) | + ve by ELISA (%) |
|---------------------------|---------------|----------------|-----------------|-------------------|
| | Type | No. of samples | | |
| Diseased Calves | Buffy Coat | 75 | 27 (36.00%) | 69 (92.00%) |
| | Nasal swabs | 75 | 19 (25.33%) | 57 (76.00%) |
| Apparently healthy calves | Buffy Coat | 25 | 0 (0.00%) | 1 (4.00%) |
| | Nasal swabs | 25 | 0 (0.00%) | 3 (12.00%) |
| Slaughtered calves | Lung tissues | 80 | 5 (6.25%) | 20 (25.00%) |

Table (3): Isolation and identification of BVDV from diseased, apparently healthy and slaughtered animals which were positive by ELISA test.

| State of animal | Sample tested | | No. of samples with CPE | Virus identification by IFAT |
|---------------------------|---------------|-----|-------------------------|------------------------------|
| | Type | No. | | |
| Diseased calves(75) | Buffy Coat | 69 | 24 (34.78%) | 24 (34.78%) |
| | Nasal swabs | 57 | 19 (33.33%) | 13 (22.81%) |
| Apparently healthy calves | Buffy Coat | 1 | 0 (0.00%) | 0 (0.00%) |
| | Nasal swabs | 5 | 0 (0.00%) | 0 (0.00%) |
| Slaughtered calves (80) | Lung tissues | 20 | 10 (50.00%) | 7 (35.00%) |

Table (3) represented the isolation of BVDV from samples positive by ELISA, out of 150 samples, 53 (35.3 %) gave signs of CPE for BVDV on the cell. These samples were then subjected for identification by indirect immunofluorescent technique (IFAT) to detect non- cytopathic BVDV, where 44 (29.3 %) were positive results (Fig. 1). MDBK cell line is considered the most common cell culture system for

virus isolation and propagation (Allam, 2000). In the present study, MDBK cells were used for three successive blind passages for samples positive by ELISA. These samples were then subjected for identification by indirect immunofluorescent technique (IFAT) to detect non- cytopathic BVDV, where 24 (26.5%) were positive results in table (3). Edwards, (1990) and Brock (1991) reported that the

Table (6): Isolation rate of mycoplasma species from examined calves Correlated to the results of Indirect haemagglutination (IHA) test on their blood sera.

| Pneumonic calves n=75 | | closely contact apparently healthy calves n=25 | |
|--|--|---|--|
| Nasal swabs | Blood serum | Nasal swabs | Blood serum |
| Recovered <i>Mycoplasma</i> sp. (%) | IHA antibodies ($\geq 1/160$) | Recovered <i>Mycoplasma</i> sp. (%) | IHA antibodies ($\geq 1/160$) |
| <i>M.bovis</i> 23/75(30.67%) <i>M.dispar</i> 7/75 (9.33%) | <i>M.bovis</i> 30/75(40.00%) <i>M.dispar</i> 9/75(12.00%) | <i>M. bovis</i> 5/25(20.00%) <i>M.dispar</i> 2/25 (8.0%) | <i>M.bovis</i> 4/25 (16.0%) <i>M.dispar</i> 3/25 (12.00%) |

Serological identification of the thirty five mycoplasma isolates that were obtained from the examined pneumonic calves by growth inhibition (GI) test revealed that the most prevalent isolated species was *M.bovis* (65.71%) followed by *M.dispar* (20.00%). These results were nearly similar to that reported by Gagea *et al.* (2006a, b) who isolated *M. bovis* in a rate of 82%. Haines *et al.* (2001) detected *M.bovis* in the lungs and joints of feedlot cattle with chronic pneumonia and arthritis. They suggested that *M.bovis* should be considered as a principal pathogen in chronic unresponsive pneumonia of feedlot cattle. Unfortunately, a total of 5 isolates (14.29%) couldn't be serologically identified and showed biochemical reactivity as glucose positive and arginine negative which may be *M.bovirhinis* or *M.bovoculi*.

In the present study, only 7 isolates out of 35 mycoplasma isolates from the examined pneumonic calves were identified as *M.dispar* (20.00%). Bitsch *et al* (1976) isolated *M.dispar* from pneumonic calf lungs in a higher percentage 56%. Moreover, Shahriar *et al.* (2002) isolated *M. bovis*, *M.dispar*, *M.arginini*, *M.bovirhinis*, BVDV, *Haemophilus somnus* and *pasteurella multocida* from the lungs of calves with chronic pneumonia.

On the other hand, the recovered *Mycoplasma species* from the apparently normal calves were only 8 isolates. The most prevalent identified species was *M.bovis* (62.50%), followed by 2 isolates (25.00%) as *M.dispar*. The least identified sp. (12.50%) was glucose positive and arginine negative. *Mycoplasma dispar* is a proven cause of pneumonia and has been reported in cases of mastitis but can be isolated from the lungs and nasal cavities of healthy and pneumonic cattle (Øystein *et al.*, 2009),

Mycoplasma species could be isolated from 20 out of 80 examined lungs in a percentage of (25.0%). These results were coincides with the findings of Byrne *et al.* (2001) who isolated *M.bovis* with ranges from 13 to 25% of pneumonic lungs of dairy and fattening herds and from 30% of calf herds with pneumonia. Also, with the results of Tend *et al.* (2004) who screened 34 cattle for the presence of

Mycoplasma species and reported that the recovery rate of *M.bovis* was 25.2 %.

In the present study, a total of 20 mycoplasma isolates were obtained from examined lung tissues. The most prevalent identified species was *M.bovis* (70.0%) followed by *M.dispar* (20.0%) and the least recovered isolate was glucose +ve and arginine negative (10.0%). This result supported by the conclusion of Nicholas *et al.*, (2000) where *M.bovis* is a major component of calf pneumonia complex. Moreover, stated that, over a third of lungs were infected with *M.bovis* while the rest contained a combination of *M.bovis* with *P.multocida* and /or *H.somnus*. Also, an alteration in the lungs were chiefly due to mycoplasma infection and the remaining bacteria contributing to complications in the pneumonic process Buchvarova and Vesselinova (1989). *M.dispar* was isolated from pneumonic calf lungs (Gourlay and Leach, 1970), which had cytopathic effect on bovine fetal tracheal organ cultures Thomas *et al.*, (1986).

Serum samples were examined by IHA test for the presence of *M. bovis* or *M.dispar* antibodies, n= number of examined calves

As shown in table (6) showed that, the indirect haemagglutinating *M.bovis* antibodies ($\geq 1/160$) were detected in the blood serum of (40.00%) of pneumonic calves higher than the recovery rate (30.67%). While the blood serum of (12.00%) of the examined pneumonic calves showed high titer ($\geq 1/160$) against *M.dispar*, the recovery rate of the organism from their nasal swabs reached to 9.33%. Gagea *et al.*, (2006 b) explained these findings that cases with specific antimycoplasmal antibodies in their serum but negative by mycoplasma isolation represented acute or primary infection. Sachse *et al.* (1993) stated that the variation between mycoplasma isolation and serological response may refer to the stage or duration of infection. Pftzner and Schimmel (1985) explained this finding that, *M.bovis* was transmitted from mastitic cows to their calves, infected their respiratory system and remained viable and infective up to sexual maturity when it could be isolated from their genital tract. Nicholas *et al* (2000) stated that using both mycoplasma isolation and

serological detection of the specific antibodies against the isolated species concurrently is expected to maximize the diagnosis of mycoplasma. Nicholas and Ayling, 2003 added that, serological detection of *M.bovis* antibodies is often a more reliable diagnostic method as the antibody levels remain high for many months. They concluded that the presence of specific antibodies indicates that the infection is invasive.

Considering the closely contact apparently normal calves, the results showed that, while the recovery rate of *M.bovis* from their nasal swabs reached to 20.0%, the detection of *M.bovis* antibodies in their blood sera was relatively lower (16.0%). In the present study, the detected IHA antibodies in the blood serum of apparently normal calves may be due to maternal immunity or latent infection due to the close contact with the diseased calves. This finding was discussed previously by Gagea *et al.* (2006b) who stated that, *Mycoplasmas* can be introduced in a herd by subclinical *Mycoplasma* carriers. These cattle shed the organism through nasal discharge for months to years without showing clinical signs. This finding is coincides with that of Jasper 1977) who stated that, in mycoplasma infected herds, it is usually to find animals have titer against mycoplasma in their serum without any history of illness. These animals may acquire partial immunity from prior exposure to mycoplasma infection. Moreover, Justice *et al* (2010) stated that, one of the currently documented routes of transmission of *Mycoplasma* spp. is through direct animal contact.

On the other hand, *M.dispar* specific antibodies were detected in the blood sera of (12.00%) out of the examined apparently healthy calves, whereas the organism was recovered from only 8.00% of their nasal swabs. Cho *et al.* (1976) observed that IHA results provided a more accurate diagnosis of

Mycoplasma herd infection than culture isolation and/or growth inhibition. They added that, IHA test is considered sensitive, reliable and highly specific and the titers were high in the infected or previously infected animals compared with the recently infected animals which may not show any titer. Nicholas and Ayling, 2003 added that, serological detection of *M.bovis* antibody is a true choice when antibiotics have been used extensively on examined herds. They stated that, animals in which *M.bovis* or *M.dispar* found only in the nasal passages without clinical symptoms were rarely seroconverting. Jurmanova *et al.* (1982) and Uhaa *et al.* 1990) stated that the isolation of mycoplasma species from the prepuce of bull or vagina of cows without clinically apparent disease, usually didn't lead to high antibody titer in their blood sera perhaps as a result of superficial localization of the o

Bacteriological results and discussion :-

Mannheimia haemolytica (*M.haemolytica*) is a gram negative cocobacilli, non-motile, non-spore forming, facultative anaerobe from the family *Pasteurellaceae*. It is a normal inhabitant of the nasopharynx of healthy animals, but it is not a normal inhabitant of the bovine lung (Abdullah *et al.*, 1992 and Rice *et al.*, 2008). As shown in table (7), 29 *M.haemolytica* (38.60%) were isolated from 75 diseased calves and 37 (46.30%) isolated from 80 lung tissues of slaughtered calves hile only 4 isolates were detected from 25 apparently healthy calves (16%), under certain predisposing factors as shipping, rearing, transportation, overcrowding, mycoplasma infection and viral infection, *M.haemolytica* may shifting from being commensally to pathogen form (Confer *et al.*, 1995).

Table (7) : Occurrence of *M.haemolytica* isolates in diseased , apparently healthy and slaughtered calves.

| State of animals | Type of samples | Total No. of sample | No. of +ve | % of +ve |
|---------------------------|-----------------------------|---------------------|------------|----------|
| Diseased calves | Nasal swabs& Blood films | 75 | 29 | 38.60% |
| Apparently healthy calves | Nasal swabs& Blood films | 25 | 4 | 16.00% |
| slaughtered calves | Lung tissues | 80 | 37 | 46.3% |
| Total | | 180 | 70 | 38.89 % |

As shown in table (8), Fifty six (80 %) of the isolated *M.haemolytica* were biotyped as biotype A and 14 *M.haemolytica* (20 %) were biotyped as biotype T table (8) . These results revealed that *M.haemolytica* biotype A was the most frequently associated with shipping fever, adisease of beef

cattle, which characterized by fibrinous pleuropnemonia , the same results were reported by Ewer *et al.*2004 and Ilhan and Keles,2007)

As shown in table (9), Antimicrobial susceptibility tests revealed that most of *M. haemolytica* biotype A was highly sensitive to

norfloxacin followed by ampicilline and erythromycin (75%, 65%, and 60% respectively) and highly resistant to ceftiofur, nalidixic acid, gentamicin, oxytetracycline, and streptomycin (90%, 85%, 75%, 65%, and 60% respectively). On the other hand most of *M. haemolytica* biotype T was highly sensitive to norfloxacin and erythromycin (92.86%, 71.43% respectively) and highly resistant to cephalaxn, nalidixic acid, oxytetracycline, and

streptomycin (85.71%, 78.57%, 64.29% and 64.29% respectively). These results were nearly similar to that mentioned by Esaki et al. (2005) and catry et al. (2005), and disagree with Mevius and Hartman (000) and Berge et al. (2006). The differences between our results and others may be attributed to many factors: misusing of antibiotics, individual physiological variation and differences in pathogenicity of the isolates and geographical localities.

Table (8): Biotyping of isolated *M.haemolytica*

| No. of total <i>M. haemolytica</i> isolated | Biotype A | | Biotype T | |
|---|-----------|----|-----------|----|
| | No. | % | No. | % |
| 70 | 56 | 80 | 14 | 20 |

Table (9): Antimicrobial susceptibility tests of *M.haemolytica* biotype A. and biotype T.

| Antimicrobial Disks | Concentration Of disk | <i>M. haemolytica</i> biotype A. | | | | <i>M. haemolytica</i> biotype T. | | | |
|---------------------|-----------------------|----------------------------------|----|-----------|-------|----------------------------------|-------|-----------|----|
| | | Sensitive | | Resistant | | Sensitive | | Resistant | |
| | | No | % | No | % | No | % | No | % |
| Ampicillin | 10mg | 13 | 65 | 9 | 64.30 | 5 | 35.71 | 7 | 35 |
| Amikacin | 30mg | 11 | 55 | 8 | 57.14 | 6 | 42.86 | 9 | 45 |
| Ceftiofur | 30mg | 2 | 10 | 7 | 50.00 | 7 | 50.00 | 18 | 90 |
| Cephaloridine | 30mg | 10 | 50 | 7 | 50.00 | 7 | 50.00 | 10 | 50 |
| Cephalexin | 30mg | 11 | 55 | 2 | 14.29 | 12 | 85.71 | 9 | 45 |
| Erythromycin | 15mg | 12 | 60 | 10 | 71.13 | 4 | 28.57 | 8 | 40 |
| Gentamycin | 120mg | 5 | 25 | 8 | 57.14 | 6 | 42.86 | 15 | 75 |
| Norfloxacin | 10mg | 15 | 75 | 13 | 92.86 | 1 | 7.14 | 5 | 25 |
| Oxytetracyclin | 30mg | 7 | 35 | 5 | 35.71 | 9 | 64.29 | 13 | 65 |
| Pencillin G | 10mg | 9 | 45 | 7 | 50.00 | 7 | 50.00 | 11 | 55 |
| Streptomycin | 10mg | 8 | 40 | 5 | 35.71 | 9 | 64.29 | 12 | 60 |
| Nalidixic acid | 30mg | 3 | 15 | 3 | 21.43 | 11 | 78.57 | 17 | 85 |

Mixed infection of BVDV, *M. haemolytica* and *Mycoplasma species*

As shown in table (10), simultaneous isolation of the three pathogens from nasopharyngeal swabs of the examined pneumonic calves was relatively high (12.00%), followed by simultaneous isolation of BVDV & *Mycoplasma sp* as well as *M.haemolytica* and *Mycoplasma sp.* (9.33%). On the other hand, there was simultaneous isolation of both BVDV and *M.haemolytica* from nasopharyngeal swabs of (8.00%) out of the examined pneumonic calves. The present isolation data of *Mycoplasma species* from examined herds based on the previous isolation of mycoplasma from these herds which may indicate that chronic mycoplasmal infection may predispose the animals to infection by other pathogens. This explanation coincides with that of (Howard et al. (1978) who stated that, the immunosuppression resulted from mycoplasmal infection enhanced the susceptibility of the animal to infection

with other microorganisms. They added that mixed infection of mycoplasmas and other microorganisms certainly lead to enhanced disease. Additionally, Shahriar et al. (2002) reported co-infection with BVDV and *M.bovis* in feedlot cattle with chronic pneumonia. They concluded that cattle with primary mycoplasmal infection undergo immunosuppression which might predispose to secondary infection with other pathogens. They suggested that the synergism between Mycoplasma and other agents may complicate the disease condition. On the other hand, Trautwein et al. (2002) concluded that mycoplasmal infection may be able at least to exacerbate a disease condition that is probably initiated by other pathogen. Although the source of respiratory viral infection is not always obvious. It is likely that a proportion of calves acquired infection from their dams early in life. Haines et al. (2001) reported that *M.bovis* was detected in the lungs and joints of 80% of cases, while BVDV and *M. haemolytica* in 40 and 23%

respectively of these cases. Significant antibody titer to *M.bovis* were detected in half of 55 pneumonic examined, of which only 7 had rising titer to viral pathogens as bovine viral diarrhoea virus (Nicholas and Ayling, 2003). However, *Mycoplasma sp.* only were recovered in a higher percentage (16.00%) than

the isolation rate of *M.haemolytica* only (9.33%) and the least recovered pathogen was BVDV only (2.67%). *Mycoplasma* infection might persist than other bacterial infection because many strains are resistant to antibiotics commonly used in the treatment of pneumonia (Thomas *et al.*, 2003).

Table (10): Simultaneous recovery of BVDV, *M. haemolytica* and *Mycoplasma species* from examined nasopharyngeal swabs and lung tissues

| Infectious agent | State of calf | | Lung tissues |
|--|------------------|------------------------|-------------------|
| | Diseased n= 75 | Apparently normal n=25 | n=80 |
| BVDV only No.(%) | 2 (2.67%) | 0(0.00%) | 0(0.00%) |
| <i>Mycoplasma sp.</i> Only No.(%) | 12(16.0%) | 5(20.0%) | 1(1.25%) |
| <i>M.haemolytica</i> only No.(%) | 7 (9.33%) | 1(4.0%) | 18(22.50%) |
| BVDV & <i>Mycoplasma sp.</i> No.(%) | 7(9.33%) | 0(0.00%) | 2(2.50%) |
| BVDV & <i>M.haemolytica</i> No.(%) | 6(8.00%) | 0(0.00%) | 2(2.50%) |
| <i>Mycoplasma sp.</i> & <i>M.haemolytica</i> No. (%) | 7(9.33%) | 3(12.00%) | 14(17.50%) |
| BVDV & <i>Mycoplasma sp.</i> & <i>M.haemolytica</i> No. (%) | 9(12.00%) | 0(0.00%) | 3(3.75%) |

Regarding the closely contact apparently normal calves, there was simultaneous isolation of *Mycoplasma sp.* and *M.haemolytica* detected in (12.00%) of examined samples. Mixed infections of *M.dispar* and *M.haemolytica* have been demonstrated in field cases of calf pneumonia (George, St. *et al.*, 1973; Fatma *et al.* (2008). The frequent association of *M.bovis* infection with *M.haemolytica* or *P.multocida*, singly or in combination, suggests synergism between these pathogens (Gagea *et al.*, 2006a and Max, 2007). They added that *M.bovis* might colonize and perpetuate the lung lesions that were initiated by *M.haemolytica* or *P. multocida*, even if infection with these pasteurellaceae are cured by antibiotic therapy and host immunoinflammatory response Robert *et al.* (2000).

On the other hand, the isolation pattern of each pathogen alone revealed that, while the *Mycoplasma sp.* could be detected in a higher percentage (20.0%) than the recovery rate of *M.haemolytica* (4.00%), BVDV couldn't be isolated from all examined apparently normal calves either alone or in combination with other pathogen.

Examination of 80 clinically pneumonic lung tissues of slaughtered calves that were collected from abattoirs revealed that, a high percentage (17.50%) of examined lung tissues colonized both *Mycoplasma sp.* and *M.haemolytica* together. This result coincides with the findings of Gagea *et al.* (2006a). On the other hand, Simultaneous isolation of the three pathogens together was detected in (3.75%) of examined lung tissues. However, simultaneous isolation of both BVDV and *Mycoplasma sp.* as well

as BVDV and *M.haemolytica* was recorded in (2.50%) of examined lung tissues. Shahriar *et al.* (2002) could detect *M.bovis* and BVDV antigens by immunohistochemical (IHC) staining in the lung tissues of calves with chronic Pneumonia in a percentage of 91% and 44% respectively. At the level of isolation of each pathogen alone from the examined lung tissues, the results revealed that, while *M.haemolytica* was recovered in a higher percentage (22.50%) than the recovery rate of *Mycoplasma sp.* only (1.25%), BVDV couldn't be recovered alone.

Pathological results & discussion:

Pneumonia is a major cause of death and economic losses to the calves industry. Recognizing the patterns of pneumonic lesions of the various types of pneumonia is important for correct diagnosis and interpretation of the lesions (Roger & Anthon, 2010). Bovine atypical interstitial pneumonia (AIP) is a multifaceted disease. It often complicated with bacterial, viral, or mycoplasmal organisms, Alan, (2010). In this work we studied the pneumonic lesions in naturally occurring bovine viral diarrhoea virus (BVDV) associated bronchopneumonia and the relationship of this condition with mycoplasma spp. infection and *M.haemolytica*. Five types of pneumonia were distinguished according to etiological agents associated with (BVDV), either single or multiple they were:

1-Caseonecrotic bronchopneumonia:

Represented 3.75% of tissue samples (3 of 80). Isolated organisms where as shown in table (12)

Mycoplasma .bovis (*M.bovis*) mixed with (BVDV) with Sever ++++

Table (11) Results of histopathological Examination demonstrate types, and percentages of pneumonia in the examined pneumonic lung tissues:

| Mixed pneumonia | | | | Non mixed | | |
|--------------------|--------------------------------|-------------------------------------|--|-------------------|------------------------------|-------------------------------|
| Type of pneumonia | Caseonecrotic bronchopneumonia | Fibrinonecrotizing bronchopneumonia | Acute and chronic fibrinosuppurative bronchopneumonia: | | Interstitial pleuropneumonia | Serofibrinous pleuropneumonia |
| Isolated organisms | <i>M.bovis</i> , & BVDV | <i>M.heamolytica</i> & BVDV | <i>M.dispar</i> & , <i>M.heamolytica</i> & BVDV | | <i>M.bovis</i> | <i>M.heamolytica</i> |
| Degree. | Sever ++++ | Less sever +++ | Moderate ++ | | | |
| No. of cases | 3/80 | 2/80 | 2/80 | 9/80 without BVDV | 6/80 | 25/80 |
| % | 3.75% | 2.50% | 2.50 % | 11.25% | 7.50% | 31.25% |

Gross lesions:

Dark red patches with interstitial edema and emphysema. In two cases there was multifocal sequestral formation in the form of circular, raised yellow foci of dry friable, caseous material. The intervening tissue in these areas was atelectatic. There was accumulation of fibrin on the pleural surface. Previous findings observed were nearly similar to those observed by Gagea *et al.* (2006 b), and coincide with those observed by Jones *et al.* (1997); Calvin *et al.* (2008); Radaelli *et al.* (2009) and Fulton *et al.* 2009)

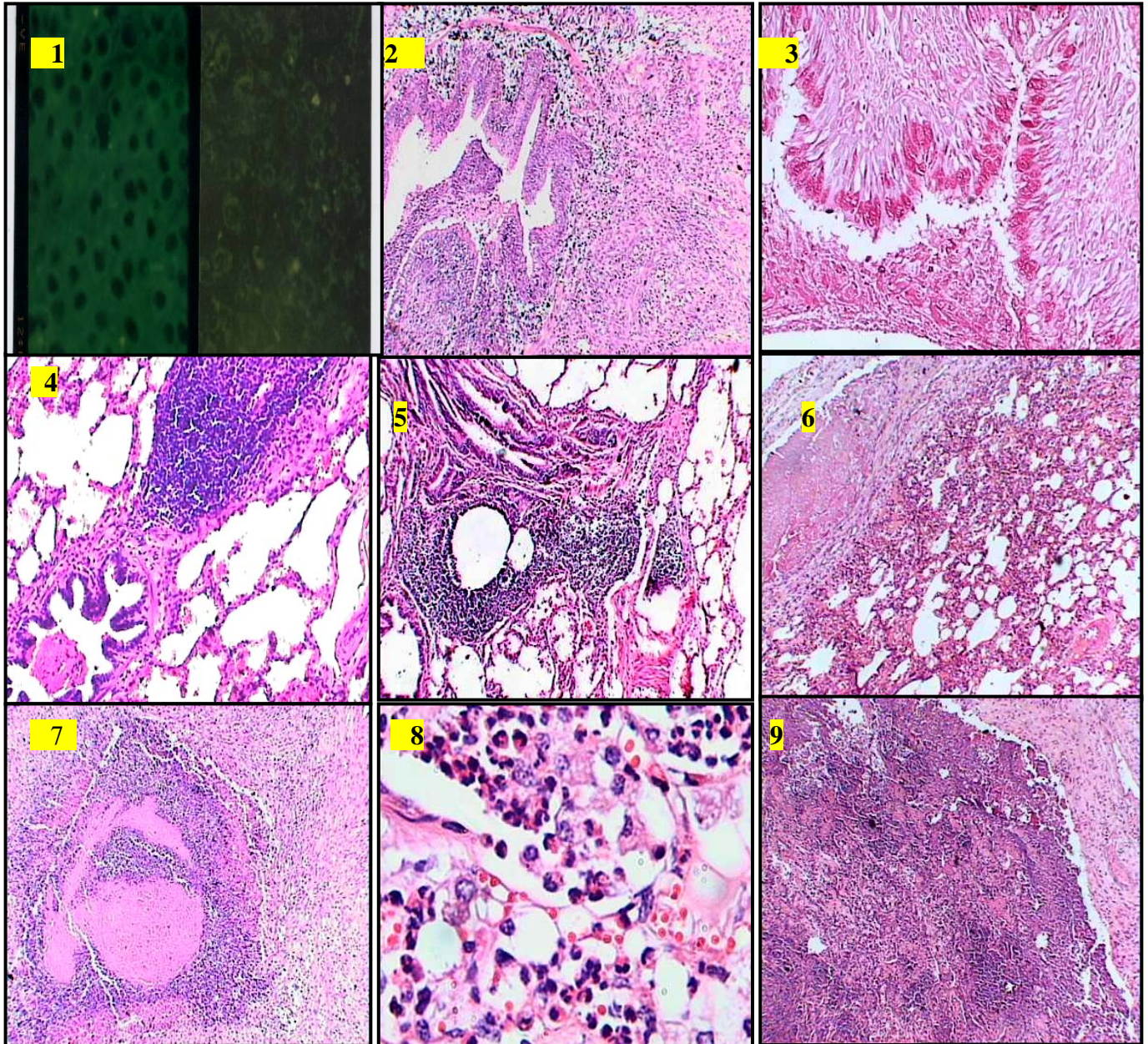
Histopathology:

The bronchiolar epithelium lining showed necrotic changes with loss of cilia or exfoliation of ciliated cells within bronchial lumen (Fig.2). Goblet cell hyperplasia also noticed and was confirmed with (PAS) stain (Fig. 3). This noticed hyperplasia considered as an inflammatory response to the development of mycoplasma specific antibodies and immune complexes resulted from persistent mycoplasmal infection. Such response acting on the respiratory epithelium and goblet cell precursors, (Zhang *et al.* 2006 and Aurora *et al.* 2006).

The peribronchial associated lymphoid tissue (BALT) showed marked proliferations and hyperplasia (Fig. 4). More over there were edema, per alveolar, perivascular, peribronchial and per bronchiolar mononeuclear cellular infiltrations (Fig.5). The Pulmonary blood vessels were engorged with blood, some of them showed fibrin thrombi with lymphocytic infiltrations, pleurisy, Interstitial pleuropneumonia (Fig. 6) with pronounced thickened alveolar walls. Previously mentioned findings were the remarkable findings of mycoplasmal infection,

were coincide with those described by (Mohamed & Abdelsalam, 2008, Fulton *et al.* 2009 and Alan, 2010). Such changes explained by Jubb *et al.* (1993); Macgavin & Zachary , (2007) as the infection which brings about immune suppression caused by BVD virus then ,ciliary dysfunction in addition to unregulated production of Tumor necrosis factor α (TNF α) barrel with the development of inflammation and increased vascular congestion so widening of vascular wall pores ,which followed by fibrin escapes from blood to pulmonary tissue under the effect of bacterial toxins and increased procoagulant activity, furthermore fibrin is chemotactic to leukocytes.

Foci of coagulative necrosis with intensely eosinophilic cytoplasm also were seen (Fig.7). These foci were delineated by a zone of neutrophils and macrophages, encircled by fibroblasts (Fig.8). Other areas showed diffuse casious necrosis (Fig.9). Mycoplasma was demonstrated with Giemssa stain in the vascular wall, interstitial tissue and inside alveoli. The latter necrotic foci which noticed microscopically was explained by Hum *et al.* (2000); Edy & Joachim, (2001) and Steven *et al.* (2003) as massive presence of *M.bovis* produce necrotizing factors as protease enzyme , hydrogen peroxide and a complex polysaccharide toxin. Necrotizing factors are resulting in destruction of the pulmonary epithelium and vascular endothelium, which leading to initiation of thrombus, ending by pulmonary infarctions with inflammatory reaction followed by severe oxidative damage of tissues. This explains the Presence of bluish green mycoplasma colonies on wall of pulmonary blood vessels.



(Fig : 1) : Normal MDBK non infected cells (A) and infected MDBK cells (notice Specific intracytoplasmic fluorescent) (B) .

(Fig : 2) bronchiole showing necrosis & exfoliation of lining cells into the lumen (H&E) x4

(Fig : 3) bronchiole showing marked goblet cell hyperplasia noticed with (PAS) x4

(Fig : 4) hyperplasia of peribronchiolar lymphoid tissue (H&E) x10

(Fig : 5) perialveolar ,perivascular , and peribronchiolar mononuclear cellular infiltrations (H&E) x4

(Fig : 6) interstitial pleuropneumonia (H&E) x4

(Fig : 7) caseous necrosis with calcifications (H&E) x4

(Fig : 8) alveoli filled with neutrophils and macrophage with congested perialveolar vessels (H&E) x40

(Fig : 9) diffuse caseous necrosis with calcifications with in center (H&E) x4

In the present study two patterns of pulmonary necrosis were noticed, the most common pattern was caseous necrosis; the second and least common pattern was coagulative necrosis. The previous findings were highly consistent with those reported by (Lopez & Marti nez, 2002; Khodakaram & Lopez 2004 and Mohamed & Abdelsalam 2008). They speculated that an original focus of coagulative necrosis progresses with time into caseous necrosis with proliferation of granulation tissue. This variation in severity, development of disease during infections may be regarded to the environmental factors (Gulliksen *et al.* 2009). Variable surface protein antigens Vsp of *M. bovis* were demonstrated in lungs of calves by (Buchenau. *et al.* 2010). In which Vsp together with immunological factors may contribute to the chronicity of pulmonary disease. The BVDV role in those lesions was due to its stimulation of cytokine hypersecretion such as interferon TNF, (Bielefeldt *et al.* 1989; Jacob *et al.* 2010).

2-Fibrino-necrotizing bronchopneumonia :

Represented 2.5% of tissue samples (2 of 80) as shown in table (12). Isolated organisms were *M. haemolytica* and *Bovine viral diarrhoea virus* BVDV. +++ With less severity.

Gross lesions:

Lung tissue was pale flabby with multiple focal grayish areas of consolidation.

Histopathology:

Bronchiolar epithelial cells showed loss of cilia and necrosis with desquamation within the lumen (Fig.10). Marked peribronchial lymphoid tissue hyperplasia was seen. Some alveolar lumens were filled with faint eosinophilic fibrin network intermingled with mononuclear cells, mainly lymphocytes, plasma cells and histiocytes (Fig. 11). Other alveoli contained cellular exudates consisted mainly of mononuclear cells. Multifocal coagulative necrosis were detected as homogenous strongly eosinophilic areas surrounded with mononuclear cells and neutrophils (Fig. 12). Oat cells and coccoid bacteria were colonized at the periphery of necrotic foci. Other foci showed congested capillaries with perivascular lymphocytic infiltration and emphysema. Pleura showed fibrinous exudate, with lymphocytic infiltrations.

The gross and microscopical findings were coordinate with (Mohamed & Abdelsalam, 2008; Jean *et al.* (2008) ; Fulton *et al.* 2009), and attributed by Jeyaseelan and Sreevatsan (2002) to the *M. haemolytica* virulence factors such as leukotoxin

which is the main virulence factor that is associated with lung lesions and secreted by all *M. haemolytica* serotypes Jeyaseelan, and Sreevatsan (2002);. At high concentrations it induces leukocyte lysis, resulting in the nuclear streaming of necrosis (Cudd *et al.*, 2001 ; Bojesen *et al.*, 2007 ; Wollums *et al.*, 2009). Another important virulence factor Lipopolysaccharide, (LPS) is synergistic with leukotoxin to induce oxidative burst of pulmonary alveolar macrophages and may inhibit the production of neutrophilic granulocyte. A polysaccharide-rich capsule is resistant to phagocytosis by neutrophils and macrophages, Also it assists in attachment, and alter neutrophil function. Furthermore Neuraminidase which increases the adhesion of the bacterium to the respiratory epithelium (Wollums *et al.*, 2009). Bovine respiratory viruses increase affinity to *M. haemolytica*, and other respiratory pathogens, by infecting ciliated epithelium and reducing mucociliary clearance. Another reason for extensive pulmonary necrosis is the secretion of maximum amounts of cytokines by alveolar macrophages, Malazdrewish *et al.* (2004); Macgavin & Zachary ,(2007).

3- Acute and chronic fibrinosuppurative bronchopneumonia:

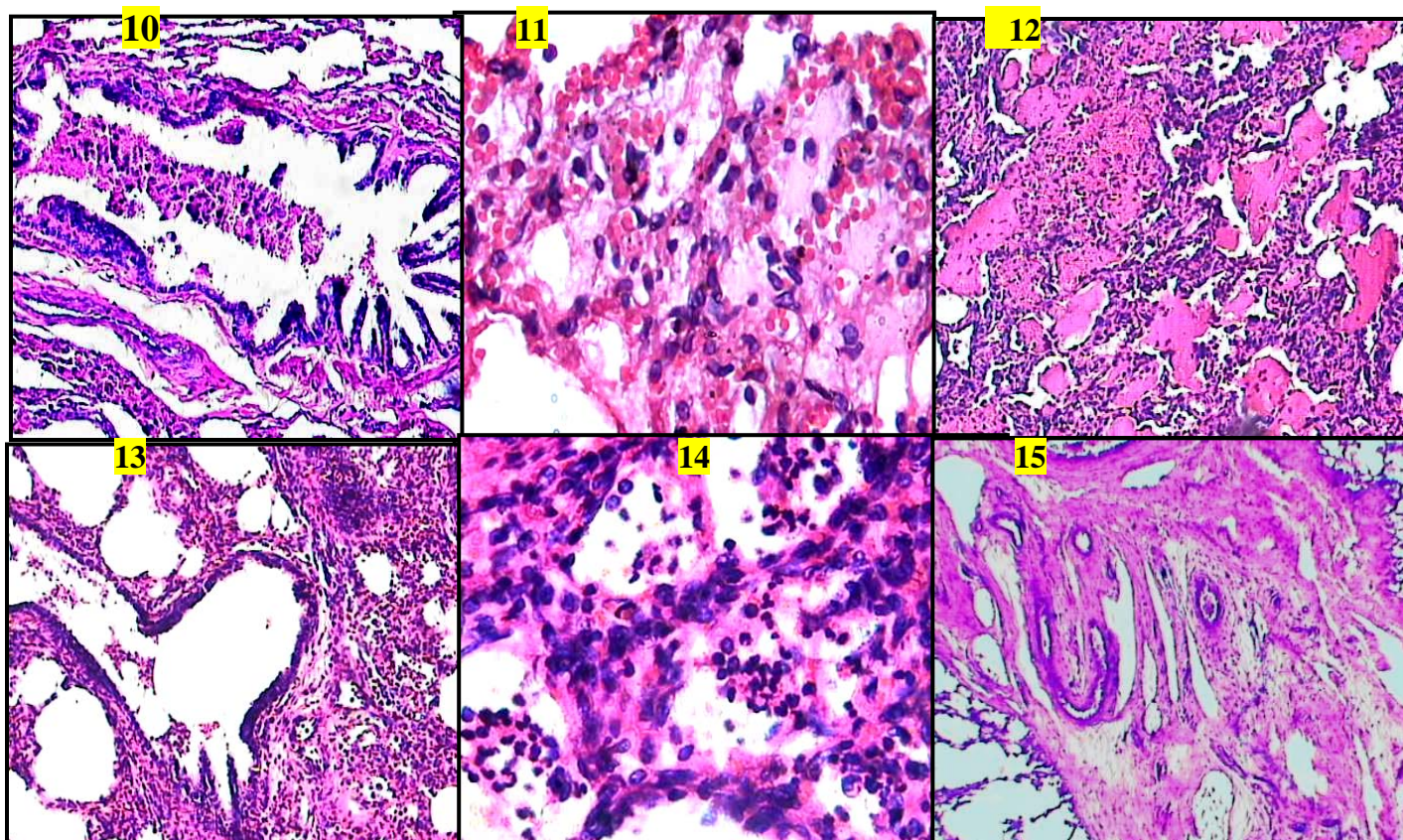
Represented 13.75% of tissue samples (11 of 80) as shown in table (12) and isolated organisms were *M. haemolytica*, *M. dispar* and (BVD). With Moderate ++

Gross lesions:

The affected areas were deep red and consolidated. Pleural showed fibrosis with fibrin adhesions was evidenced. Pulmonary parenchyma showed foci of suppuration irregularly shaped and, delineated by a grayish rim.

Histopathology:

Bronchiolitis, bronchiectasis were seen in most cases of this category (Fig. 13) Alveoli showed atelectasis and necrosis with macrophage , neutrophils , fibrin, or both, and streaming of nuclear chromatin to form oat cells, foci of coagulative necrosis typical of *M. haemolytica* pneumonia(Fig. 14). Pleura showed aggregations of neutrophils with edema and congestion. A process of chronic fibrosis was seen with granulation tissue formation (Fig: 15). Within interstitial tissue histiocytes, plasma cells and lymphocytes were also noticed.



(Fig :10) Bronchiolar epithelial lining showing loss of cilia and necrosis with desquamation into the lumen (H&E) x10

(Fig :11) alveolar lumen with exudates of fibrin network intermingled with mononuclear cell mainly histiocytes ,lymphocytes , plasma cells and ,monocytes (H&E) x40

(Fig : 12) multifocal coagulative necrosis as homogenous strongly eosinophilic areas infiltrated with mononeuclear cells (H&E) x10

(Fig :13) Bronchiolar necrosis, intrabronchiolar neutrophils, bronchiolitis (H&E) x10

(Fig :14) Alveoli showing necrosis with neutrophils, and streaming of nuclear chromatin to form oat cells. (H&E) x40

(Fig :15) Pleura showing thickening with fibrous and granulation tissue proliferation (H&E) x10

Those neutrophil influx with alveolar necrosis and minimal bronchiolar necrosis were specific picture to *M. dispar*, and were agreed with Linda & Reggio, (2002); Jeyaseelan, and Sreevatsan (2002); Ajuwape *et al.* (2003); Ewer and Wieler (2004); Ilhan & Keles, 2007 and Fulton *et al.* (2009). Leukocytes within the alveoli and bronchioles undergo necrosis but retain a ghost-like outline with hyper-eosinophilic cytoplasm and fragmented nuclei (Caswell and Archambault, 2008). Studies using *M. dispar* indicate the pathogenesis of *Mycoplasma* pneumonia in calves involves degeneration and impairment of ciliated respiratory epithelial cells, thereby predisposing the lung to secondary infection with additional pathogens (Almeida & Rosenbusch,

1994). Coinfection of *M. hemolytica* with viruses resulting in complex infection after long-distance transportation and coldness (Jean *et al.* 2008).induced a moderate increase in the lesion severity (Gourlay &Houghton, 1995). But only when calves were infected with *M. bovis* prior to infection with *M. hemolytica*. *M. bovis* pneumonia in calves is typically more severe when multiple pathogens, including *M. hemolytica*, (Bucharova & Vesselinova, 1989; Gregg *et al* 2010).

4-Non mixed pneumonia:

A- Interstitial pleura- pneumonia:

Represented 7.5 % (6 of 80) cases and isolated only mycoplasma species, lung tissue

showed interstitial lobar pneumonia, thickening interstitial tissue, desquamations of bronchiolar epithelium with in lumen. In addition to perivascular ,perialveolar ,lymphocytic cuffing those findings coincide with those observed by,(Rodriquez *et al.*, 1996).Those lesions regarded to mycoplasma toxins.

B- Serofibrinous pleuropneumonia :

Found in 31.25% cases, represented 25/80 and the isolated organism was only *M.haemolytica* This type represent the higher percentage, this illustrated by (Thomas *et al.*2002;Caswell and Williams 2007) as *M. hemolytica* are the most common organisms commensally of the bovine nasopharynx which, during stress can overcome host defense mechanisms establishing infection in the lower respiratory tract .Lung tissue showed widespread accumulation of, fibrin mash intermingled with neutrophils, macrophages . Airway Lumina and interlobular septae were distended with serofibrinous exudates. Those observations were caused by *M. hemolytica* toxins and nearly similar to those of (Larsen *et al.* . 2001).

4. Conclusion

Viral agents are usually considered the primary pulmonary pathogen, capable of destroying the respiratory epithelial lining to a degree allowing other agents to colonize. In addition to acute BVDV infections or persistent postnatal BVDV infections, undergo immunosuppression through destroying of immune competent cells of the host. The latter increases the susceptibility of these animals to secondary bacterial infection and increasing pathogenicity of concomitant diseases, that is why mycoplasmal and manhaemial infection may able to exacerbate a disease condition and initiated by other pathogen. Other theory suggested that, the viral and mycoplasmal agents are the primary infections and the bacterial gents are there as the secondary invaders.and these two suggestions supports our view.

Corresponding author

Hanaa, A. Ghoneim
Animal Reproduction Research institute (ARRI)
Giza, Egypt
*hanaeg2002@yahoo.com

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