

**A COINCIDENTAL CASE: THE PATHOMORPHOLOGICAL FINDINGS OF PNEUMONIE WITH DIFFERENT AGENTS IN A COW**

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**ABSTRACT**

Tuberculosis and parastic infestations, which be composed commonly of hydatidosis and lungworms, are taken part in lesions of lower respiratory system disorders. Tuberculosis is a contagious and zoonose disease due to *Mycobacterium* sp. Inflammatory changes characterized by caseification necrosis are encountered in either exudative or productive form. Cystic hydatidosis is constituted by *Echinococcus* sp. as developmental form of the parasite in intermediate hosts primarily cattle, sheeps, goats and almost all mammalians. It cause cystic structures on mainly lungs and subsequently different tissues and organs. The inflammatory reaction which reacted to the cystic structures is often encapsulated. Verminouse pneumonie is generally developed by larval form of specific worms pertaining to family of *Metastrongylidae*, which lead to some clinical signs such as chronic progressive cough and respiratoric distress. Inflammatory cell infiltrations are localized in interstitial tissue especially between interalveoler septa, bronchi and bronchioles. It is encountered with hemorrhagie and necrosis in initial phase, through larval migration with inflammatoric changes or sometimes granulomatouse reaction in late phase are seen interstitiel tissue.

In the case, it was aimed that tuberculosis and lesions due to cyst hydatid and lungworm infestations were described macroscopically, histopathologically and histochemically in lungs of a 2.5 years old Holstein cow. Granulomatous reaction were encountered in tuberculose and cyst hydatid lesions in lungs, although the reaction were not completely developed against larvae. The remarkable point of the case is coincided with three type of lesions, of which become in different periods of animal's lifetime on same lobes.

**Key words:** *cow, lung, tuberculosis, hydatidosis, lungworm*

**INTRODUCTION**

Respiratory disease cause serious problems not only for breeders but veterinarians also because the disease make considerable economical losses for not allowing to provide measurements and treatments. Respiratory disorders are highly important as regard health of animal and economical deficits for breeders because are common symptoms growth retardation and no producing of tuberculosis, cyst hydatid, lungworm infestions (Caswell and Williams 2008; Panuska, 2006; Yıldız, 2006; Eckert et al., 2001; Soulsby, 1986). In this contex, lungs are mainly effected organs due to anatomical and histological features, especially in cattle (Belkhiri et al., 2009). Particullarly, it points it out to give an important exactly when taken into consideration for one of most encountered disease in ruminants of Turkey (Vural and Alcigir, 2010; Acıöz et al., 2008; Esatgil and Tüzer 2007; Gurler, 2006). *Myobacterium bovis* known as bacils of classical tuberculosis cause infection primarily in cattle and respectively in deer, gazelle, bison, waterbuffalo, sheeps, goats, llamas, horses, pigs, elephants, rhinoceros, dogs, cats, foxes, minks and badgers); *Mycobacterium tuberculosis* (primarily in human and in order of pigs, monkeys, cats, dogs, cattle and parrots); *Mycobacterium avium* (primarily in aviary birds, cattles, pigs, horses, sheeps and monkeys). However, the tuberculosis are mostly encountered in cattle, and especially in young animals between these (Thoen et al., 2009; Caswell and Willaims, 2008; Shitaye et al., 2006; Sevcikova et al., 1999; Borlow et al., 1989). The disease is widely spread through aerosol or droplet infection into respiratory tract and respectively alimentary, congenitally, cuteneous, and via genital tract and iatrogenic applications while that made of contaminated material or drug in the

intramammal injections (Caswell and Williams, 2008). Most lesions of tuberculose are localized on caudodorsal lobes and subpleura of diaphragmatic lobes in lungs (Adeniran et al., 1992).

On the other hand, parasitic disease are second important and widespread pulmonary disorders of ruminants. In both Turkey and many countries of the world, cyst hydatid disease or hydatidosis are responsible for zoonotic disease. The disease are caused by *Echinococcus* sp. especially, *E. granulosus* and *E. multilocularis*, one of which is *E. granulosus* that lead to unilocular hydatidosis. Adult forms of the *E. granulosus* are found as enteric parasites in carnivores (dogs, wolves, coyotes, foxes and dingos). The eggs of parasites are spread into environment and these are generally taken via oral route. The oncospheres are removed from the parasitic eggs and larval forms localize in mainly lungs, liver, and rarely heart, spleen, muscle and bones. Larval form of parasite cause to cyst hydatid in these tissues and organs (Daryani et al., 2007; Sayek et al., 2004; Eckert et al., 2001). Cyst hydatid, which caused by *E. granulosus* is a unilocular pouche filled with colorless fluid. Cystic wall is mainly composed of three layer. There are also numerous scolex, brooding capsules and fluid having antigenicity in lumina of cyst. Inner side, there is a germinative membran producing to scolex. In next layer, cuticular membran, which is acellular and has semipermeable characteristics (no transferring of bacteria). In outer side, is found that pericyst or known as adventitial layer, be produced by host. Moreover, the pericyst is a fibrous capsule which composed of fibroblasts and fibroblasts. On the other hand, cyst hydatid is restricted by inflammatory reaction with variable intensity. In the reaction, in which is organized by host, there are mononuclear cells, epithelioid histiocytes and foreign body giant cells and sometimes neutrophilic leucocytes (Daryani et al., 2007; Hakverdi et al., 2009).

The another parasitic infestation of lung is lungworms infestation. Even so, infestations fewer incidence than that of other respiratory disease of cattle lungworms have second important etiological agent between parasites that cause to verminous pneumonie (Belkhir, 2009). Some species of lungworms are localized in paranchym of lung although most of them are found in bronchi. Metastrongylidae family is primarily agent of lungworm (Haziroglu, 2001). In lungworm infestation of cattle is most encountered type is *Dictyocaulus viviparus* (*D. viviparus*) from *Dictyocaulus* sp. Larval forms (L) of nearly all parasites release off while the eggs are found in lungs. L1, which set of free, go firstly into alimentary tract and then throw them out via fecal route. Larvae are cast off two times. And L3, which has capability of infestation, are taken by oral route while young cattle as define host graze in pasture. Infective form of the larvae penetrate into wall of intestine. The larvae migrate into nearest mesenteric lymph nodes of ileum, caecum and colon. Then, larvae cast off in third times. Finally, L4 arrive to lung by passing through by lymphatics and vessels. Larvae cast off in fourth times and become as L5. Then, the adult form of larvae go away into bronchi and trachea. In this period, the parasite lead to verminous pneumonie on lungs (Panuska, 2006; Caswell and Williams, 2008).

In the case, the lesions, which be incidentally developed, cyst hydatid and lungworm infestations were described macroscopically and histopathologically. In general, productive type or granuloma were characterized with inflammatory cellular reaction. The histopathological diagnose were confirmed by other histochemical stainings, Ziehl-Nielsen were preferred for tuberculosis, Periodic Acid Schiff (PAS) for parasites and Alizarin Red for dystrophic calcification in old productive form of lesions.

## MATERIAL AND METHODS

The material of the case that compose of showing different characteristics and sizes in lesions, which localized on lungs in a 2.5 year old Holstein cow. After macroscopical examination, tissue samples were taken from lesions for histopathology and fixed in 10 % formalin. Then, tissue samples were processed routinely and embedded in paraffin. Sections were cut at 5 $\mu$  thickness from paraffin blocks and stained with hematoxylin-eosin (H&E). After routine histopathological evaluations, the histochemical procedures were performed for confirmation of diagnosis. It was

applied for sections Ziehl-Nielsen (Z.N) for tubercle bacilli, Periodic Acid Schiff (PAS) for cyst of echinococcus and also Alizarin Red for detection of dystrophic calcification especially in old granuloma of every kind of lesions.

## RESULTS

Macroscopically, it was observed that some restricted foci had 0.5-4 cm in diameter. The lesions were intensified on the left side lobus cranialis and caudalis, diaphragmatic and accessory lobes of lungs. The foci had generally outgrowth from surface of lung and partially embedded. The foci had firm and yellowish-gray and cut section were caseified in lung. On the other side, encapsulated cysts, which were diametered in 0.5-1 cm, were localized on left caudal and diaphragmatic lobes. The cysts were also excessive and had fluctuant consistency which filled with clear fluid. Almost all lobes were hyperemic, emphysematic and collapsed (Figs. 1-3). Microscopically, large caseification necrotic areas, were attended to contain also cellular debris, which were at center of tubercles. At periphery of necrotic area, it was arranged fewer neutrophilic leucocytes, macrophages, lymphocytes, plasma cells, epithelioid histiocytes and Langhans type giant cells. In outside, there were fibrous capsule restrict the all reaction and necrotic area. In some of tubercles, it was attended that dystrophic calcification areas accumulate onto necrotic areas Hematoxylin and Alizarin Red (Figs. 4-6). Moreover, tubercle bacilli were detected by Ziehl-Nielsen staining method and the mycobacterium were found in cytoplasm of macrophages and also giant cells or cell free in some areas (Fig. 7).

In addition to these tubercles, some larval forms of lungworms were localized on both lumina of bronchioles and alveoli. The parasites were surrounded by lymphocytes, macrophages, only a few numbers plasma cells, neutrophilic leucocytes and eosinophilic leucocytes (Fig. 8-9). In some microscopical foci, noticed that cyst, which had cuticular and laminar walls, were restricted by inflammatory reaction foreign body giant cells, macrophages, epithelioid histiocytes, lymphocytes and lymphoblasts in order. In outside, there were fibrous capsules, which composed of fibrocytes and fibroblasts, The appearance of inflammatory reaction was found of likely to tubercles (Fig 10). Cystic wall was stained as PAS positive. Other findings which composed of hyperemia in capillaries and arteriols, emphysem and atelectasis were not conspicuous. In addition, there was filled with mucinous secret in some lumina of bronchioles and goblet cells. Along with these, BALT were hyperplastic at periphery of bronchus.

## DISCUSSION

In respiratory disease of cattle, tuberculosis is most common cause of pneumonia and subsequently a part that cyst hydatid and lungworm infestations as parasitic disease (Belkhir, 2009). Tuberculosis is a both zoonotic and infectious disease caused by Mycobacterium sp. Most spread types is mycobacterium bovis and M.tuberculosis, even though there are found apathogenic and saprofitic types of Mycobacterium at environment. In the disease, respiratory tracts and lungs are affected in cattle. In addition, retropharyngeal and mediastinal lymph nodes are affected also (Biberstein and Hirsh, 1999; Corner et al., 1990). The etiological agent infect the animal by mainly aerosol, and respectively oral route, congenital, percutaneous, genital and iatrogenic (applications of contaminated drugs intramammary) (Caswell and Williams, 2008). Primary lesions were frequently found on caudodorsal lobes and diaphragmatic lobes subpleura (Adeniran et al., 1992). Macroscopically, the morphological lesions can be changeable in exudative and productive forms. In exudative form, small or large areas of lungs were covered with purulent necrotic with typical appearance. In spite of this, restricted or encapsulated foci which contain caseification necrosis, are seen in grayish-white color in various size. Sometimes, mineralizations in productive form can be accorded to the lesions (Caswell and Williams, 2008). Macroscopically, caseification necrosis, be sorted as a subtype in coagulation necrosis and necrotic debris, mononuclear cells, epithelioid histiocytes and Langhans type giant cells are found in productive form of lesions. It sometimes can

be encountered in with localizations of neutrophile leucocytes of just perihper of necrotic areas and mineralizations. In general, the necrotic areas and inflammatory cell reactions are limited by fibrous capsule. In exudative form, there are necrotic or live cells and inflammatory cells are found in large casefication necrosis. Tubercule bacills can be found as cell free or in cytoplasm of macrophages and Langhans type giant cell (Palmer et al., 2007; Thoen and Barletta, 2004; Neil et al., 1994). In the case, tuberculosis was noted in productive form as mentioned in literatures before. However, dystrophic mineralizations were more intensive in same areas. It was thought that tuberculosis was getting old and developed for long time ago. On the other hand, it was attended that numerous lymphocytes and macrophages were dense against cysts. But, the reaction did not show the typical characteristics of productive form, which is reported in literatures. Particullarly, there were no encapsulation and dystrophic mineralization. For these hence, it was thought that the reaction would be able productive form in following period for this lesions. Moreover, it was encountered with larval form of parasite which is one of lungworm, was thought to be Dictyocaulus sp. at perihper of this areas, numerous inflammatory cell infiltrations were intensified at interalveoler interstitial region. The cell infiltrations contain to lymphocytes in huge proportions, and macrophages and few eosinophile leucocytes. In addition, alveol and broncioler wall were destructed in some microscopical areas. For the last mentioned finding, mechanic effects are reported to be developed by being constrained of parasites in some documents (Caswell and Williams, 2008; Panusca 2006; Haziroglu, 2001). On the other hand, other finding is hyperplastic changes of BALT, bronchioler epitheliums and peribronchioler musculer layer (Haziroglu, 2001). In this regard, the findings were accorded to literatures. However, it was not seen that productive type inflammation (epitheloid histocyte, giant cell and fibrouse capsule) were developed as to be in verminouse pneumonie. Many bronchioles had hyperplastic epitheliums and BALT. Goblet cells were fullled with secrets, despite of no seeing in hyperplasia of bronchioler muscle cells.

As a result, it was thought that the lesions, which composed of tubercles, was in different developing stage and was coincided with cyst hydatid and lungworm infestations in some cases. It is made a desicion that the all lesions were developed in different period of animal's life. It is believed that the results from case will provide the important supports to this subject, to which was carried out in future.

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## Figures

**Figure-1:** Macroscopical appearance of tubercule caseified areas (arrow) and encapsulation (arrowhead)

**Figures-2 and 3:** Macroscopy of cyst localized in left caudal lobe (arrows)

**Figure-4:** Granulomatous inflammation. Caseification necrosis (star), inflammatory cell infiltration (arrow) and fibrous capsule (arrowhead), x40, HE.

**Figure-5:** Inflammatory cell infiltrations composed of Langhans type giant cell (arrow), mononuclear cells and rarely neutrophilic leucocytes, x100, HE.

**Figure-6:** Dystrophic calcification of central area of tubercles (star), x.100, Alizerin Red staining,

**Figure-7:** Tubercle bacilli (arrows), x400, Ziehl-Nielsen.

**Figure-8:** Larval forms of lungworm (arrow) in alveoli with interstitial pneumonie and tubercule (star), x40, HE.

**Figure-9:** Larval forms of lungworms in lumina of alveoli and bronchioli (star) and hyperplasia of BALT, x100, HE.

**Figure-10:** Granulomatous reaction against cyst (arrows) and lungworms, x100, HE.

