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Pharmacological Studies on Tildipirosin in Calves

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Abstract

Bovine respiratory disease (BRD) is the primary health problem in the beef cattle industry worldwide. Tildipirosin was injected as a metaphylaxis to healthy animals and also as a therapeutic to the clinically diseased animal at a dose of 4 mg/kg B.W. TD is effective in reducing the mortality rate and increasing the recovery rate from *P. multocida* infection which induces damage to the bronchioles and alveoli with fibrinopurulent bronchopneumonia represented by dilated bronchiole with caseated material in its lumen associated with severe leukocytic cells infiltration in the wall, multifocal areas of necrosis organized exudate infiltrated with many neutrophils in alveoli. PCR is considered the test of choice in the diagnosis of *Pasteurella* as it can identify organisms at any level regardless of tiny quantities of bacteria's genome, consequently, the sensitivity and specificity of the test increased. Tildipirosin injection caused no significant changes in RBC count after treatment for the treated healthy and treated diseased group compared with the control group. Tildipirosin showed no significant changes in hemoglobin content and HCT of the treated healthy group but a significant decrease in TD treated diseased group was revealed post-treatment compared to the control group. Single subcutaneous injection of Tildipirosin causes an important decrease in MCV, and MCH levels in TD treated diseased group and decreasing in the MCHC of TD treated healthy group at day 7 compared to the control group. Tildipirosin causes no significant changes in Malondialdehyde (MDA) levels in TD-treated healthy while it increased in the TD-treated diseased group at all days after treatment compared to the control group. No significant changes occur in the superoxide dismutase (SOD) level of TD treated healthy group and TD treated diseased group compared to the control group. In conclusion, *P. multocida* is one of the most prevalent causes of BRD in Egypt, and tildipirosin is highly effective as a prophylactic and metaphylactic treatment against BRD cases caused by *P. multocida*, and it has a potentially anti-inflammatory effect.

KEYWORDS
Bovine respiratory disease, cattle, Tildipirosin.

INTRODUCTION

Bovine respiratory disease (BRD) is the most public and fiercest disease in calves, especially in recently transported ones. BRD generally refers to undifferentiated fever in addition to several respiratory signs. It is a multifactorial complex disease as it is caused by a mixture of environmental, viral, and bacterial causes like *Mannheimia haemolytica*, *Histophilus somni*, and *Pasteurella multocida*. Bovine Respiratory disease in calves causes great economic losses for the dairy and beef industry worldwide either directly through increased mortalities or indirectly through a decrease in the ADG, feed efficiency, the performance of calf's beef, loss of weight of affected animals, negative effect on carcass composition and costs of prophylaxis and metaphylaxis treatments (Griffin, 2010; Panciera and Confer, 2010; Horwood and Mahony, 2011; Griffin, 2014; Joshi *et al*., 2016).

Pasteurellosis happens in the whole world, but it is a specific problem in the tropics due to heat and humidity as environmental stress is a significant cause of the disease complex. *Pasteurella multocida* is recognized as the key pathogen in the BRD complex and it is the most famous microorganism identified in respiratory disease affecting young cattle which are recently weaned or highly stressed. *P. multocida* can be diagnosed by several methods including clinical examination coupled with bacterial isolation on selective media, PCR, immunohistochemistry, and gross and histopathological lesions which are supportive in the early diagnosis of *Pasteurella*. However, isolation of *Pasteurella* is not easy as it is a fastidious organism and despite being able to grow well on blood and chocolate agar, it can be overgrown by other microbiota in sputum. Moreover, it resembles other Gram-negative bacteria and can be easily misidentified (Townsend *et al*., 1998; Rocke *et al*., 2002; Kabeta *et al*., 2015; Amin, 2020).

Macrolides are broad-spectrum antibiotics that have been accepted in cattle for treatment, control, and prevention of BRD, they act by binding reversibly to the 23S ribosomal RNA in the 50S subunit of bacteria, inhibiting mRNA that directed synthesis of protein and inhibited bacterial growth. These antibiotics are well-dispersed into cells, tissues, and organs. Moreover, they can accumulate in some tissues and have a long half-life. Also, Macrolides have an unrivaled ability to pile up in lung tissues, viewing quick and wide distribution besides existence in pulmonary epithelial lining fluid (PELF), so it is used especially in respiratory system infections. Macrolides may show bactericidal effects when they reach high concentrations in respiratory system infections

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(Abugharbieh *et al*., 2004; Yazar Soyad Bir *et al*., 2018).

Tildipirosin is the latest generation of macrolide antibiotic used in veterinary medicine, It is a semi-synthetic antibiotic derived from the naturally occurring compound tylosin. TD is recently administered to pigs and cattle for the treatment of respiratory diseases caused by certain bacteria, it is authorized by the EMA for the treatment and prevention of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *M. bovis* infections (Menge *et al*., 2012; Abu-Basha *et al*., 2020).

This study aimed to isolate and identify the bacterial cause of respiratory disease in calves, evaluate the tildipirosin efficacy on BRD clinical signs, and study its value in the treatment of bovine respiratory disease in calves, and also, evaluating the pharmacodynamic effect of tildipirosin on the hematological parameters and liver function tests.

MATERIALS AND METHODS

Drug

TEDO - Each mL of TEDO 18% contains 180 mg of tildipirosin. Manufactured by ADWIA CO. S.A.D.10th Ramadan city, Egypt. Dose: 4 mg/kg (1 mL/45 kg body weight) s/c injection (Evans, 2005; EMA, 2011).

Experimental calves

This study was conducted in 2000 Holstein calves their age ranged from 6 to 8 months that were imported from Spain and introduced to their farm in the Wady Elnetron area, Egypt. Their body weight ranged from 180 to 250kg. Calves were divided into 2 groups; clinically diseased group (suffer from BRD) and a healthy group. Out of them, thirty Holstein calves with an average body weight of 60-70 Kg were divided into 3 groups; the 1st group was kept as a control group, the 2nd diseased group was injected with TD at a dose of 4mg/kg B.W subcutaneously and the 3rd treated healthy group was injected with TD at a dose of 4mg/kg B.W subcutaneously at a special dairy farm at Dakahlia governorate.

Experiment using animals in this study were conducted according to The guidelines of Mansoura University, Egypt.

Samples

Collection of blood samples

Two blood samples (the first sample for hematological and the second sample for biochemical studies) had been collected from each animal in the three groups (control, diseased, and treated healthy group) at zero days, after 3, 7, 14 days and after 28 days from the drug injection. The first blood samples were collected on Wassermann tubes that contain EDTA (1 mg /ml blood) from the jugular vein of all calves for hematological parameters studies (erythrocytes count, leucocytes count, Hb, PCV, MCV, MCH, MCHC, thrombocyte count) and MDA. The second blood samples were collected in Wassermann tubes without anticoagulant from the jugular veins of all calves and allowed to clot at room temperature. The serum was separated by centrifugation at 3000 rpm for 15 minutes; the sera were collected in 1.5 ml Eppendroff tubes and kept frozen at -20˚C for biochemical studies (Total proteins, albumin, globulin, and ALT, AST, ALP, and SOD) according to Stoffregen *et al*. (1997).

Collection of lung samples for histopathological examination and PCR assay

Samples were collected from the diseased part of the lung that showed gross lesions from dead calves that suffer from signs of pneumonia according to standardized procedures and good laboratory practices to sidestep the sample contamination and confirmed the quality of the data. Lungs' specimen had been stored in separate sterilized freezer bags at -80°C until analysis by PCR assay. Specimens from similar parts had been fixed in 10 % buffered formalin for 48 h, then dehydration of the sample was done by passing it in a graded alcohol sequence after that the sample was immersed in paraffin wax. Cut sections (3.5 µm) had been obtained from each sample and had been stained with hematoxylin and eosin (HE) for histopathological examination (Bancroft *et al*., 1996).

Molecular identification of Pasteurella species by using uniplex PCR

Pasteurella sp. was identified according to the methods described by Sambrook *et al*. (1989).

Histopathological examination of lung specimens

it was performed according to Bancroft *et al*. (1996).

Hematological and Biochemical analysis

Total erythrocytes count (RBCs), hemoglobin concentration (Hb), hematocrit blood test (HCT), mean corpuscular volume, (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PLT), total leukocytes count (WBCs) were determined by the hematological analyzer as previously described by Feldman *et al*. (2000). Serum total proteins were determined spectrophotometrically according to the method described by Pagana and Pagana (2010). Albumin was determined colorimetrically by using the dye-binding technique with bromocresol green and the A/G ratio was calculated by dividing the albumin value by the globulin value according to Fischbach and Dunning (2009). Serum globulin was determined by the differences between total protein and albumin according to Chernecky and Berger (2008). Serum samples were used also for the determination of aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using the special kits according to the method described byHare (1950); Pagana and Pagana (2010) respectively. Plasma L- Malondialdehyde (MDA) was determined colorimetrically according to the method adopted by Esterbauer *et al*. (1982). superoxide dismutase (SOD) activity in each serum was detected according to the method described by Packer and Glazer (1990).

Statistical analysis

Data obtained in this study were statistically analyzed by independent T-test, using the SPSS computer program (version. 20) (SPSS, 2015).

RESULTS

Clinical examination of calves under experiment

Calves showed respiratory manifestation, high respiratory rates, while 150 animals out of 220 calves showed cough, 90-95

animals manifested by oculonasal discharges. However, mouth breathing appeared in 40 animals, and 170 animals suffered from fever as shown in Table 1. The rate of infection increased day by day, the mortality rate before injection reached to 150 cases but after injection eby xactly 72 h, it decreased to only 10 cases as shown in Table 1.

Molecular identification of Pasteurella species by using uniplex PCR

Four lung samples were examined for the presence of *Pasteurella* species DNA by using uniplex PCR, results revealed that *Pasteurella multocida* was recovered from all samples as shown in Figure 1.

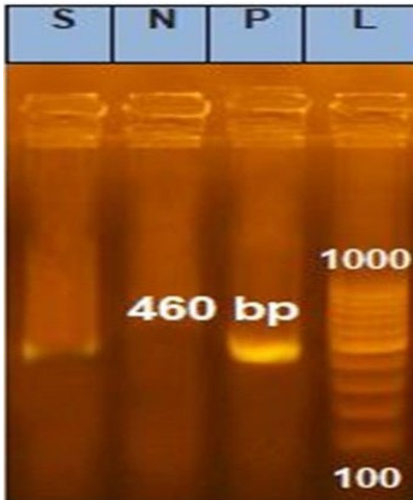

Fig. 1. PCR products on an agarose gel stained by Ethidium Bromide following electrophoresis, positive *Pasteurella multocida*, at 460bp.

Gross examination of lung samples

Gross examination during lung necropsy revealed consolidation of pulmonary lobs with severe congestion and blood oozing from the surface as shown in Figure 2.

Histopathological examination of lung sample

Histopathological investigation showed damage of bronchioles and alveoli with fibrinopurulent bronchopneumonia represented by dilated bronchiole with caseated material in its lumen associated with severe leukocytic cells infiltration in the wall, multifocal areas of necrosis organized exudate infiltrated with many neutrophils in alveoli as shown in Figures 3 and 4.

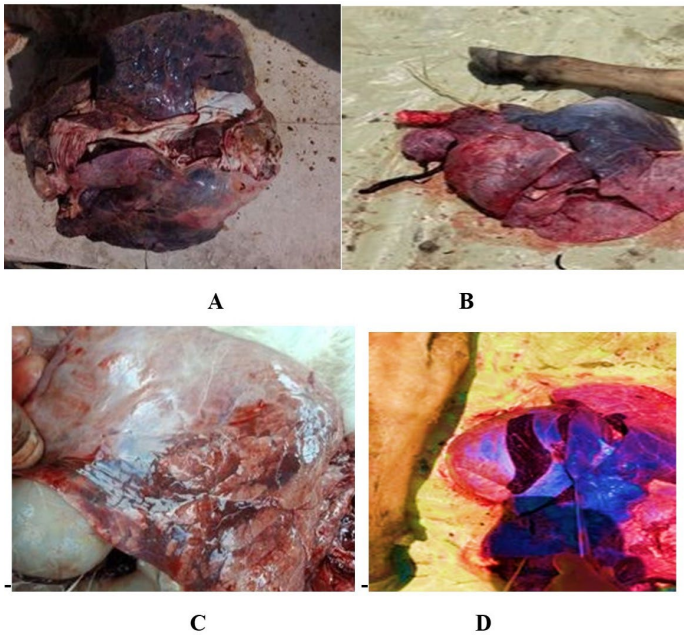

Fig. 2. Gross lesion of calf lung (Pasteurellosis): Showing red to grey consolidation of pulmonary lobs with hemorrhage (A-B) and severe congestion(C) with blood oozing from the cut surface of pulmonary lobs (D).

Hematological and biochemical findings

In this study, results showed no significant changes in RBCs count in the tildipirosin-treated healthy and diseased group at zero, 3, 7, 14 and 28 days after treatment compared to the control group (Table 2).

Also, the present work reflected no significant changes in Hb content in the treated healthy group, but a significant decrease ($p < 0.05$) in TD treated diseased group at zero, 7, 14 and 28 days and significant increase only in the 3rd day post-treatment compared to the control group (Table 2). Moreover, the obtained results revealed a significant decrease ($p < 0.05$) in HCT in TD treated diseased group with no significant changes in the treated healthy group at zero, 3, 7, and 28 days after treatment compared to the control group (Table 2).

MCV showed a significant increase ($p < 0.05$) in TD treated healthy group at zero day only after treatment compared to the control group, while it showed a significant decrease ($p < 0.05$) in TD treated diseased group at zero, 3, 7, 14 and 28 days post-treatment compared to the control group (Table 2). MCH significantly decreased ($p < 0.05$) in TD treated diseased group while there were no significant changes in TD treated healthy group at zero, 3, 7, 14, and 28 days post-treatment compared to the control group (Table 2). Moreover, results showed a significant decrease in MCHC level on day 7 of TD treated healthy

Table 1. Clinical examination of the investigated calves.

Total number of animals (n.=2000)				
Diseased animals		Healthy animals	Dead animals	
n.=220		n.= 1630	Before injection	After injection
Clinical signs	No.			
Cough	150		150	10
Nasal discharge	90			
Rapid respiration	220			
Ocular discharge	95			
Mouth breathing	40			
Temperature	170			
Recovered cases	210	1630	Total dead	160

group post-treatment compared to the control group, while it revealed a significant increase ($p < 0.05$) in TD treated diseased group at zero, 3,14 and 28 days post-treatment compared to the control group (Table 2).

There was a significant decrease ($p < 0.05$) in the total leucocytic count at zero and 14 days of the treated healthy group however it showed a significant increase on days 7 and 28 post-treatment in the treated healthy group and days 0, 7, and 28 of the treated diseased group after treatment compared to the control group (Table 2).

No significant change was observed serum total protein level of TD treated healthy group. However, it revealed a decrease ($p < 0.05$) at zero, 3, 7, 14 and 28 days post-treatment of the TD-treated diseased group compared to the control group (Table 3).

No significant change was detected in serum albumen level in TD treated healthy group, but a decrease ($p < 0.05$) was detected in TD treated diseased group at zero, 3, 7 and 28 days after treatment compared to the control group (Table 3).

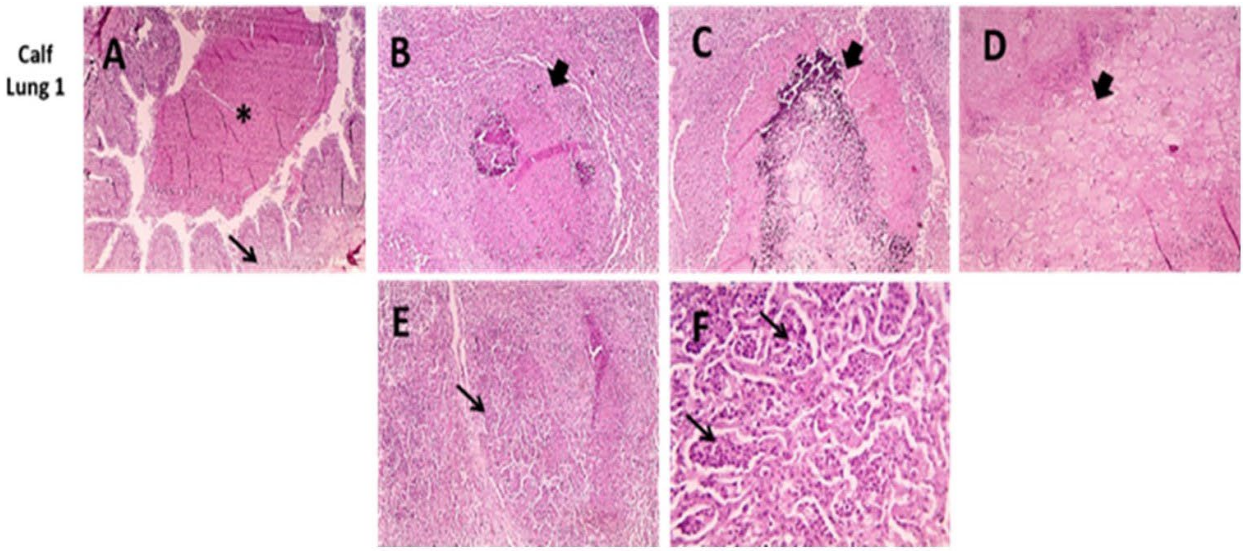

Fig. 3. Microscopic pictures of HE-stained lung sections from calf Pasteurellosis showing dilated bronchiole with caseated material in its lumen (*) associated with severe leukocytic cells infiltration in the wall (thin arrows) (A), multifocal areas of necrosis (thick arrow) (B-C-D) with the presence of organized exudate infiltrated with many neutrophils in alveoli (thin arrow) (E&F). Low magnification, 100 µm (A-B-C-D-E), high magnification, 50 µm (F).

Table 2. Hematological parameters of control and Tildipirosin treated calves.

		Days (before and after treatment)				
Parameters	Groups	Zero day	3rd	7th	14th	28th
Hb (g/dl)	Control	12.73±0.49^{a}	8.36±0.26^{b}	13,25±0.32^{a}	13.43±0.21^{a}	13.40±0.23^{a}
	Treat. healthy	12.85±0.47a	7.41±0.19^{b}	12,60±0.44a	12.70±0,14a	12.55±0.19a
	Treat. diseased	8.20 ± 1.06^{b}	10.98 ± 1.34^{a}	10.61 ±1.30^{b}	10.70±1.39^{b}	10.23±1.45^{b}
RBCs (x10^{6}/µl)	Control	5.38±0.18^{a}	5.21±0.18^{a}	5.35±0.29^{a}	5.71±0.27^{a}	6.20±0.33^{a}
	Treat. healthy	6.53±0.41^{a}	6.01±0.34^{a}	5.78±0.33^{a}	5.95±0.26^{a}	6.26±0.24^{a}
	Treat. diseased	5.19±0.67a	6.39±0.77^{a}	6.31±0.86^{a}	6.28±0.98^{a}	5.83±0.96^{a}
HCT (%)	Control	40.31±1.11^{a}	40.26±0.93^{a}	41.35±1.09^{a}	40.70±0.92^{a}	41.96±1.12^{a}
	Treat. healthy	42.85±1.64^{a}	42.31±1.79^{a}	42.96±1.54^{a}	41.91±1.34^{a}	41.48±1.80^{a}
	Treat. diseased	23.10±2.65^{b}	26.76±3.35^{b}	26.25±3.71^{b}	26.00±4.33^{a}	23.80±4.03^{b}
MCV (fl)	Control	84.70±1.70^{b}	85.35±1.94^{a}	85.25±2.01^{a}	85.91±2.12^{a}	85.26±2.19^{a}
	Treat. healthy	89.26±1.17^{a}	88.83±0.98^{a}	89.40±0.91^{a}	90.03±1.00^{a}	90.83±1.04^{a}
	Treat. diseased	42.40±0.53^{c}	41.86±0.39^{b}	41.51±0.32^{b}	41.16±0.42^{b}	40.70±0.32^{b}
MCH (pg)	Control	30.00±1.26^{a}	30.93±1.23^{a}	30.63±1.57^{a}	30.76±1.47^{a}	31.18±1.43^{a}
	Treat. healthy	29.96±0.77^{a}	31.21±0.82^{a}	31.46±1.16^{a}	32.01±1.20^{a}	32.56±1.10^{a}
	Treat. diseased	16.01±0.61^{b}	17.15±0.58^{b}	16.91±0.59^{b}	17.28±0.53^{b}	17.85±0.66^{b}
MCHC (g/dl)	Control	34.10±0.66^{b}	35.20±0.65^{b}	39.90±0.86^{a}	33.81±0.43^{b}	34.76±0.59^{b}
	Treat. health	34.68±0.54^{b}	34.98±0.55^{b}	34.06±0.79^{b}	35.50±0.41^{b}	35.81±0.41^{b}
	Treat. diseased	38.08±1.92^{a}	41.21±1.76^{a}	40.96±1.74^{a}	42.25±1.68^{a}	44.10±1.88^{a}
Platelets (x10^{3}/µl)	Control	232.33±24.85^{b}	241.00±24.67^{b}	240.83±19.85^{b}	252.500±20.79^{b}	196.66±23.99^{c}
	Treat. healthy	248.66±20.48^{b}	271.83±12.00^{b}	275.83±8.46^{b}	287.83±5.81^{b}	260.66±9.66^{b}
	Treat. diseased	601.66±70.90^{a}	692.66±88.48^{a}	753.22±88.16^{a}	628.66±46.80^{a}	576.83±67.34^{a}
WBCs (x10^{3}/µl)	Control	5.20±0.37^{b}	7.53±0.21^{a}	5.95±0.41^{c}	6.31±0.54^{a}	5.73±0.38^{b}
	Treat. healthy	4.88±0.24^{c}	8.51±0.45^{a}	6.63±0.49^{b}	5.21±0.64^{b}	7.06±0.55^{a}
	Treat. diseased	6.65±0.40^{a}	9.55±0.79^{a}	7.30± 0.20^{a}	6.15±13.32^{a}	6.15±0.16^{a}

The data showed no significant changes in globulin level in TD treated healthy group while it showed a significant increase ($p < 0.05$) in the TD treated diseased group at 14 and 28 days after treatment compared to the control group (Table 3)

There was a significant increase in serum ALT activity in TD treated healthy group at zero day only with no significant changes after treatment compared to the control group. While ALT activity showed a significant decrease ($p < 0.05$) in TD treated diseased group at 3, 7, and 28 days after treatment compared to the control group while it showed (Table 3). These results showed no significant changes in serum AST activity of TD treated healthy group, but a significant increase ($p < 0.05$) was recorded in TD treated diseased group on all days after treatment compared to the control group (Table 3). On the other hand, no significant change in serum ALP activity was observed in TD treated healthy group, but mirrored a significant increase ($p < 0.05$) in the ALP activity in TD treated diseased group at all days after treatment compared to the control group (Table 3)

The TD-treated healthy group showed no significant changes in MDA level but revealed a significant increase ($p < 0.05$) in its level in the TD-treated diseased group at all days after treatment compared to the control group (Table 3). Meanwhile, there was no significant changes in the SOD activity of TD treated diseased group and TD treated healthy group in all the days post-treatment compared to the control group (Table 3).

DISCUSSION

Bovine respiratory disease (BRD) is the primary health problem in the beef cattle industry worldwide and has a serious animal welfare impact that causes great economic losses. The rate of this complex disease is strictly linked to the predisposing factors, involving the severity of stress factors, the immune status of the calf, environment, facilities, microbial factors, and previous management of health. Over the past two decades, BRD has shifted from being near fatal to a curable disease. A significant improvement in the prognosis of this disease has been attributed to the long-term use of macrolides. Before the use of macrolide therapy, the prognosis of diseased animals with BRD was extremely poor despite numerous treatment alternatives (Compiani *et al*., 2014).

The current study was conducted in 2000 Beef calves were imported from Spain. Out of them, 220 calves suffered from respiratory manifestations; some cases showed high respiratory rates owing to hypoxia and dyspnea that could be due to severe inflammation in alveoli and bronchioles that restrict the exchange of gases and respiration, while 150 animals out from the 220 diseased calves showed cough, 90-95 animals had oculonasal discharges that may be attributed to inflammatory changes in the nasal mucus membrane. However, mouth breathing appeared in 40 animals which may be attributed to the reduction in the lung capacity and weight besides bronchiectasis which caused because of chronic lung infection, and 170 animals suffered from fever as shown in Table 1. The rapid treatment is mandatory and should be early before lung consolidation, which may hinder the treatment. Consequently, TD was injected as a metaphylaxis to the apparently healthy animals and also as a therapy to the clinically diseased animal at dose 4 mg/kg B.W., the mortality rate before injection reached to 150 cases while after injection by exactly 72 h, it decreased to only 10 cases as shown in Table 1, these results confirmed the effectiveness of TD in treatment of the disease and in decreasing the mortality rate and increasing the recovery rate which agreed with Confer *et al*. (2016) and Celestino *et al*. (2020). Moreover, the immunomodulatory mechanisms of TD beside anti-inflammatory effects by decreasing cytokine levels, inhibiting secretion of mucous, inhibiting virulence of the bacteria and inhibiting effects of viral infection might explain the benefit in mortality reduction (Zalewska-Kaszubska and Górska, 2001; Kanoh and Rubin, 2010). The apparently healthy animals were followed for the appearance of new clinical cases of BRD. While the clinically diseased animals were followed for clinical recovery.

P. multocida can be diagnosed by several methods including clinical examination coupled with bacterial isolation on selective media, PCR, immunohistochemistry, and macroscopic and microscopic lesions which are supportive in the early *Pasteurella* diagnosis. Several challenges were faced during the isolation of *Pasteurella* from the field. Firstly, despite being able to grow

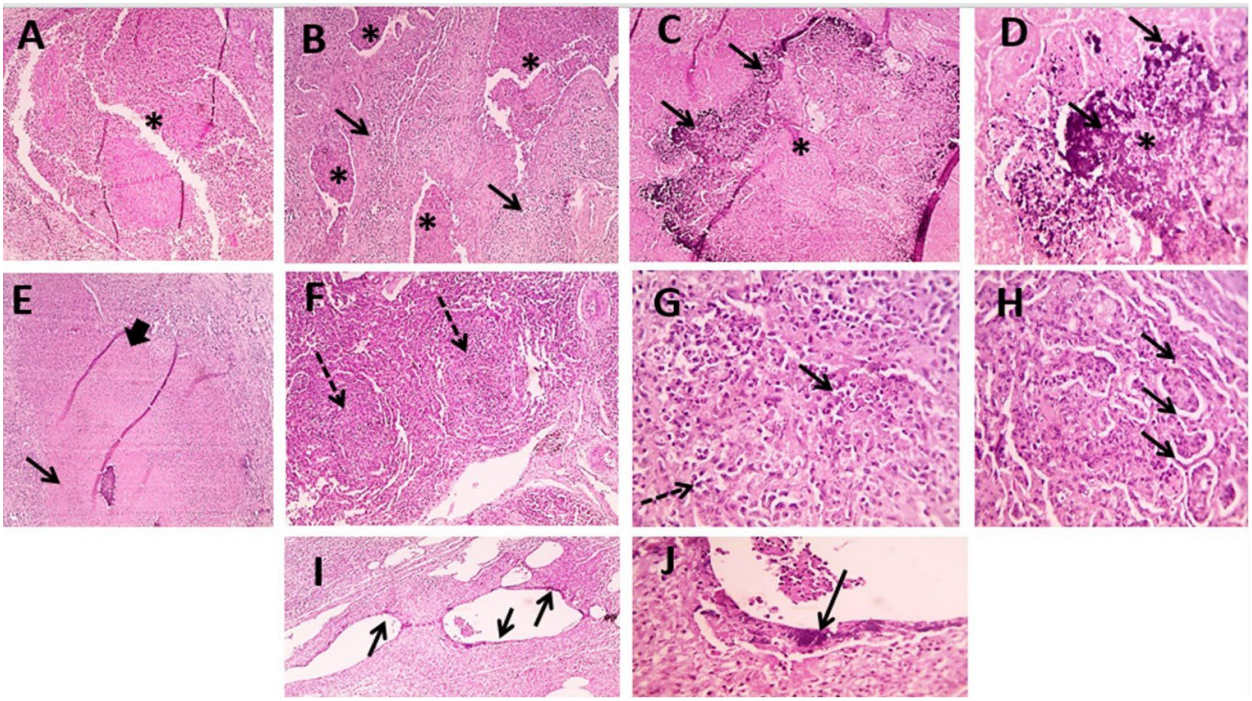

Fig. 4. Microscopic pictures of HE stained lung sections from calf 2 (Pasteurellosis): showing dilated bronchioles with caseated material in its lumen (*) (A) associated with severe leukocytic cells infiltration in the wall (thin arrows) (B), multifocal areas of necrosis (*) with suppuration (thin arrows) (C-E). Multiple granuloma-like nodules are seen (F) (dashed arrows) consisting of macrophages, epithelioid cells (dashed arrows), and neutrophils (straight arrows) in the center (G). An organized exudate infiltrated with many neutrophils is seen in the alveoli (thin arrows) (H). Moreover, necrosis and calcification of bronchiolar epithelium are seen (thin arrows) (I&J). Low magnification, 100 µm (A-B-C- E- F- I), high magnification, 50 µm (D-G- H- J)

well on blood and chocolate agar, *P. multocida* can be overgrown by other microbiota in sputum. Moreover, it resembles other Gram-negative bacteria and can be easily misidentified. In addition, the samples in this study were collected from Wady ElNetron farm which was so far from the laboratory, so the samples were susceptible to deterioration and contamination. Therefore, samples were frozen, the diagnosis of pasteurellosis was dependent on post-mortem and microscopical examination as well as PCR. These methods were faster and more accurate than other traditional methods in the detection of such fastidious organisms, in addition to being more appropriate to the frozen samples we used.

Generally, PCR is considered the test of choice in the diagnosis of *Pasteurella* as it can identify organisms at any level regardless of tiny quantities of bacteria's genome, consequently, the sensitivity and specificity of the test increased. The histopathological investigation is also a very good way to diagnose *Pasteurella* as it decreases the time consumption for diagnosis. In the current study, the red-to-grey consolidation of pulmonary lobs with hemorrhage and severe congestion with blood oozing from the cut surface of pulmonary lobs were the most prevalent gross lesions was detected in the lungs of most cases which were in accordance with what has been reported by previous studies (Attia *et al*., 2016; Amin, 2020).

The histopathological investigation revealed dilated bronchiole with caseated material in its lumen associated with severe leukocytic cells infiltration in the wall, multifocal areas of necrosis organized exudate infiltrated with many neutrophils in alveoli, also revealed a varying degree of fibrinopurulent and suppurative bronchopneumonia as shown in Figures 3 and 4, these results agreed with Attia *et al*. (2016) and Amin (2020).

The most popular trait of acute pulmonary infections caused by *P. multocida* is the fibrin-suppurative and necrotic inflammatory reactions. The respiratory tissue is infiltrated with a huge amount of fibrin, neutrophils, erythrocyte, and zero-proteinaceous material. In addition, extensive parenchymal necrosis was noticed. These changes could be attributed to the endotoxins and toxic proteins of the *P. multocida* such as polysaccharide, lipopolysaccharide, and leukotoxin. also, these changes might be due to the effect of inflammatory factors that had been produced by neutrophils and other inflammatory cells (Amin, 2020). Furthermore, it was found that the existence of neutrophils in the exudate of the respiratory tissue in the present study is due to the role of neutrophils in the clearance of *P. multocida* from the respiratory tissue as they are a part of the innate immune response. Additionally, multifocal coagulative necrosis was noticed to be a characteristic pulmonary lesion that is always related to *P. multocida* antigens (Amin, 2020). The results of PCR confirmed the diagnosis of *Pasteurella multocida* in the lung samples.

In this work, Tildipirosin injection caused no significant changes in RBC count at zero, 3, 7,14, and 28 days after treatment for the treated healthy and treated diseased group compared with the control group and these results are the same in tildipirosin and other macrolides like tilmicosin and Tylvalosin which cause no significant changes in RBCs count after single subcutaneous injection in sheep, horses and broiler chicken (DiK *et al*., 2018; Abu-Basha *et al*., 2021; Shwaish *et al*., 2021), In contrast to other macrolides that cause a significant decrease in RBCs count (Altunok *et al*., 2002; Yazar *et al*., 2004; Elsayed *et al*., 2014; Bedeer *et al*., 2021).

Tildipirosin showed no significant changes in Hb content and HCT of the treated healthy group but a significant decrease in the TD treated diseased group was revealed post-treatment compared to the control group as shown in (Table 2), these results are opposite to those obtained by other researchers who mentioned no significant changes in Hb and HCT in diseased groups after single subcutaneous and intravenous injection of Tildipirosin and other macrolides in sheep, rabbit, Bulb mice, Holstein

Table 3. Some biochemical parameters of control and Tildipirosin treated calves.

		Days (before and after treatment)				
Parameter	Groups	Zero-day	3rd	7th	14th	28th
ALT (IU/L)	Control	28.66±1.89^{b}	26.33±1.89^{a}	28.83±1.77^{a}	26.50±1.92^{a}	26.00±1.65^{a}
	Treat. healthy	31.16±1.01^{a}	28.83±0.79^{a}	30.83±0.87^{a}	28.50±0.76^{a}	25.83±1.19^{a}
	Treat. Diseased	16.83±4.49^{b}	11.33±4.58^{b}	23.33±0.47^{b}	21.83±0.39^{b}	22.33±0.66^{b}
AST (U/L)	Control	29.500±1.40^{b}	27.16±1.35^{b}	29.16±1.22^{b}	31.50±1.40^{b}	31.66±0.91^{b}
	Treat. Healthy	29.00±0.85^{b}	26.33±0.80^{b}	28.16±0.87^{b}	30.16±1.40^{b}	31.83±1.49^{b}
	Treat. Diseased	63.33±12.91^{a}	51.500±8.03^{a}	86.00±17.16^{a}	81.83±22.85^{a}	74.33±13.96^{a}
ALP (U/L)	Control	501.66±64.10^{b}	503.33±61.300^{b}	516.66±59.19^{b}	496.66±53.45^{b}	486.66±53.45^{b}
	Treat. Healthy	516.66±58.17^{b}	518.33±55.28^{b}	530.00±54.09^{b}	515.00±54.69^{b}	503.33±54.44^{b}
	Treat. Diseased	2390.33±54.38^{a}	2094.33±39.82^{a}	4343.33±127.51^{a}	1777.33±43.97^{a}	1681.50±52.6^{a}
Total protein (g/dl)	Control	5.35±0.44^{a}	5.03±0.36^{a}	5.30±0.36^{a}	5.28±0.35^{a}	5.00±0.36^{a}
	Treat. healthy	5.30±0.33^{a}	5.05±0.33^{a}	5.35±0.31^{a}	5.61±0.33^{a}	5.21±0.39^{a}
	Treat. Diseased	3.43±0.15^{b}	3.48±0.13^{b}	3.63±0.16^{b}	5.93±0.45^{a}	4.08±0.13^{a}
Albumin (g/dl)	Control	3.62±0.23^{a}	3.35±0.18^{a}	3.47±0.14^{a}	3.30±0.11^{a}	3.17±0.13^{a}
	Treat. healthy	3.65±0.20^{a}	3.41±0.17^{a}	3.33±0.16^{a}	3.78±0.14^{a}	3.45±0.17^{a}
	Treat. Diseased	1.96±0.05^{b}	1.98±0.04^{b}	2.01±0.11^{b}	3.50±0.27^{a}	2.36±0.04^{b}
Globulin (g/dl)	Control	1.73±0.07^{a}	1.68±0.09^{a}	1.83±0.07^{a}	1.98±0.06^{b}	1.83±0.06^{b}
	Treat. Healthy	1.81±0.07^{a}	1.76±0.06^{a}	1.95±0.06^{a}	1.15±0.07^{b}	1.03±0.05^{b}
	Treat. Diseased	1.46±0.11^{a}	1.50±0.09^{a}	1.61±0.06^{a}	2.43±0.18^{a}	2.71±0.09^{a}
MDA (nmol/ml)	Control	29.50±0.95^{b}	31.66±1.14^{b}	32.33±0.80^{b}	32.66±0.55^{b}	31.16±0.87^{b}
	Treat. healthy	30.16±0.94^{b}	31.66±1.14^{b}	32.66±0.61^{b}	33.66±0.42^{b}	31.50±0.42^{b}
	Treat. Diseased	54.26±3.18^{a}	58.18±4.64^{a}	58.05±4.14^{a}	277.33±5.48^{a}	258.16±3.55^{a}
SOD (U/ml)	Control	265.00±12.58^{a}	268.500±12.77^{a}	273.33±13.33^{a}	264.50±10.51^{a}	258.33±9.09^{a}
	Treat. Healthy	268.33±9.09^{a}	278.50±9.19^{a}	286.66±9.54^{a}	275.00±8.46^{a}	266.83±8.29^{a}
	Treat. Diseased	266.66±6.26^{a}	268.33±9.17^{a}	281.16±9.99^{a}	254.76±3.46^{a}	251.86±2.74^{a}

calves, Broiler chickens and horse (Altunok *et al*., 2002; Yazar *et al*., 2004; Elsayed *et al*., 2014; DiK *et al*., 2018; Abu-Basha *et al*., 2021; Shwaish *et al*., 2021)

While these results were compatible with Gheith *et al*. (2015) and Said *et al*. (2016) who found that there was a significant reduction in Hb level after tilmicosin treatment in diseased groups in broiler chicken and mice, also it matched with Bedeer *et al*. (2021) who reported a significant decrease in HCT after tilmicosin treatment in calves. The subnormal levels of RBC parameters and indices (measures of number, size, and hemoglobin concentration) showed that macrolide treatment led to acute anemia in a dose-dependent manner. decreased percentage of its hematocrit value indicated bone marrow dysfunction and/or blood loss (Gheith *et al*., 2015).

Decreased levels of Hb and PCV in the treated diseased group could be a consequence of adaptation and compensatory processes reacting to chronic pulmonary disease and long-lasting hypoxemia in animals that suffered from chronic respiratory diseases together with RBCs destruction by micro-organisms secretions (Ramadan *et al*., 2019).

Single subcutaneous injection of Tildipirosin caused significant decreases in MCV, and MCH levels in TD treated diseased group and decreased the MCHC in TD treated healthy group at day 7 compared to the control group. Other macrolides give the same results as Tilmicosin injection in mice caused significant decreases in MCH and MCHC (Gheith *et al*., 2015) and also Tilmicosin treatment in Holstein's calves decreased the level of MCV (Bedeer *et al*., 2021), at variance to this study no significant changes or even significant increases in MCV and MCH was noticed in other studies after subcutaneous injection of Tildipirosin and other macrolides in mice, horses and Holstein's calves (Yazar *et al*., 2004; Elsayed *et al*., 2014; Abu-Basha *et al*., 2021; Shwaish *et al*., 2021). Moreover, Bedeer *et al*. (2021) reported a significant increase level of MCH after tilmicosin treatment in calves.

A significant increase in MCHC level in TD treated diseased group in 0, 3,14, and 28 days in this study agreed with Bedeer *et al*. (2021) who mentioned a significant increase in MCHC after tilmicosin treatment in calves. RBC size is expressed by the mean corpuscular volume (MCV) and usually reflected the grade of regeneration from anemia so in this study decrease in MCV correlate with microcytic RBC and so indicates iron-deficiency anemia but MCV is usually not taken as an isolated measurement, rather, it was likened to the results of your other RBC indices and CBC values, like hemoglobin and hematocrit, MCH and MCHC to confirm the diagnosis and help determine the cause. MCV is a measurement of the average size of your red blood cells. MCH results tend to mirror MCV results. This is because bigger red blood cells generally contain more hemoglobin while smaller red blood cells tend to have less (Gheith *et al*., 2015). MCH and MCHC both are indicators of hemoglobin health in the blood and the low level of them in this study indicated hypochromic microcytic anemia, this condition means that RBCs are smaller than usual and have diminished levels of Hb.

Tildipirosin caused a significant increase ($p < 0.05$) in platelets count of TD treated healthy group at 28 days and TD treated diseased group at zero, 3, 7, 14, and 28 days post-treatment compared to the control group, these obtained results were similar to those recorded after tilmicosin administration in Holstein's calves (Bedeer *et al*., 2021). Otherwise, Tildipirosin and Tilmicosin treatment in horses, mice, and broiler chickens respectively did not change platelet count (Yazar *et al*., 2004; Abu-Basha *et al*., 2021; Shwaish *et al*., 2021).

The fact that platelet count increased following macrolide treatment may be attributed to the antibacterial and immunomodulatory effects of macrolides as platelets are considered guards of the vascular system due to their huge quantity in the circulation together with the high range of functional immunoreceptors that they expressed. the platelet–bacteria interplay is a complex game as Platelets express a wide range of potential bacterial receptors, and bacteria bind these receptors either directly, or indirectly via fibrinogen. Platelets were also important for neutrophil formation as they secrete immune modulators which are chemotactic for neutrophils and lymphocytes this may explain the increase in the level of neutrophils and lymphocytes that we will reveal later in our study. Moreover, microcytic anemia as we explained previously is the main cause of the decreased level of MCV and MCH in our study also it is a common cause of increased platelet levels (Hamzeh-Cognasse *et al*., 2015).

Tildipirosin injection caused a significant decrease ($p < 0.05$) in the total leucocytic count of the treated healthy group at zero and 14 days but a significant increase was revealed at 7 and 28 days of treated healthy group and at 0, 7 and 28 days of the treated diseased group after treatment compared to control group (Table 2), the same findings obtained by other macrolides as Tilmicosin that caused a significant increase in the total leukocytic count after injection in calves (Bedeer *et al*., 2021), in contrast to this study, tildipirosin and tilmicosin injection in horse, sheep, and mice showed no significant changes in WBCs count or even decrease after tylosin and Tilmicosin injection in chicken (Yazar *et al*., 2004; Elsayed *et al*., 2014; Said *et al*., 2016; DiK *et al*., 2018; Abu-Basha *et al*., 2021; Bedeer *et al*., 2021; Shwaish *et al*., 2021)

Results also detected no significant changes in the total protein and albumen level of TD treated healthy group at zero, 3, 7, 14 and 28 days while it significantly decreased ($p < 0.05$) in the TD-treated diseased post-treatment compared to control group. It has been reported that other macrolides have caused a similar effect in rats and broiler chicken (Jordan *et al*., 1999; Elsayed *et al*., 2014). However, in other studies, Tildipirosin and other macrolides didn't change the level of total protein and albumen in sheep and Holstein calves respectively (DiK *et al*., 2018; Bedeer *et al*., 2021). Furthermore, the present study detected no significant changes in the globulin level of the TD healthy treated group while a significant increase has been noticed in TD treated diseased group at 14 and 28 days post-treatment and this was confirmed by Bedeer *et al*. (2021). The recorded hypoproteinemia and hypoalbuminemia with hyperglobulinemia are usually recorded due to the anorexia of the diseased animals together with the liver failure to manufacture protein. Also, may be due to the bacteria and their toxins which increase the permeability of the blood capillaries causing the escape of the protein (Attia *et al*., 2016). The substantial increase in globulin in BRD affected cases in this research was thought to be due to the immune system stimulation as a result of the infectious agents (Ramadan *et al*., 2019).

Tildipirosin injection in the current study revealed a significant increase of ALT activity in TD treated healthy group and no significant changes in AST and ALP activities of the treated healthy group while it showed a significant decrease ($p < 0.05$) in serum ALT activity with a significant increase ($p < 0.05$) in serum AST and ALP activities in the treated diseased group, the similar effect had been reported previously by other macrolides, tilmicosin cause a significant increase in ALP activity after treatment in calves (Bedeer *et al*., 2021), also a significant increase in ALP and AST activities after tilmicosin treatment in mice were recorded (Gheith *et al*., 2015). In contrast, tilmicosin caused an increase in the activity of ALT in rats (Jordan *et al*., 1999), moreover, no significant changes were noticed in all ALT, AST, and ALP activities after tilmicosin and tildipirosin treatment in broiler chicken, sheep, and New Zealand rabbits (Altunok *et al*., 2002), also, no significant changes in both AST or ALT activities were reported by Bedeer *et al*. (2021).

ALT considered as a cytoplasmic enzyme so increasing its activity in the treated healthy group indicated mild injuries in the liver caused by the drug. While AST is considered a mitochondrial enzyme so the high activity of it in plasma in the treated diseased group reflects severe hepatic tissue injury, moreover, increasing the activity of AST with BRD was usually due to increased respiration together with muscle work throughout a long period which usually occurs in severe cases of respiratory disease. ALP is formed mostly in the liver, however, it is nonspecific to hepatic injury as it is formed by other tissues like the kidney, placenta, and bone. Nevertheless, its increase along with AST and ALT may

refer to that its source of elevation is hepatic (Gheith *et al*., 2015; Ramadan *et al*., 2019).

Tildipirosin caused no significant changes in MDA level in TD-treated healthy at zero, 3, 7, 14, and 28 days while it increased ($p < 0.05$) in TD treated diseased group at all days after treatment compared to the control group. The macrolides' effect on antioxidant status had been reported before. Tilmicosin, another macrolide antibiotic, increased the level of MDA in Balb mice Yazar *et al*. (2004), also it was found that azithromycin and Tulathromycin increased ($P < 0.05$) the levels of serum MDA in rats and rabbits (Er *et al*., 2011; Atli *et al*., 2015), in contrast, erythromycin, azithromycin, roxithromycin, and clarithromycin decreased the level of MDA (Aktan *et al*., 2003). A variation in macrolides effects on the oxidative status might be attributed to the alterations in dose or to the drug's molecular structure (Er *et al*., 2011), The cause of the increased level of MDA may be attributed to that macrolides are metabolized by the liver. Therefore, it might have achieved a high concentration in the liver and caused changes in MDA concentrations, moreover, it was found that macrolides suppress the release of reactive oxygen species and have antioxidant effects by increasing the superoxide dismutase enzyme (DiK *et al*., 2018).

A balance between the production of reactive oxygen species (ROS) and antioxidants like SOD was developed in normal physiological conditions. If this balance is disrupted by the overgeneration of ROS and/or inadequate antioxidant capacity oxidative damage had been developed, subsequently oxidative damage causes lipid peroxidation and MDA is the end product of lipid peroxidation so measurement of MDA is accepted as a basic test of lipid peroxidation in clinical settings worldwide (Er *et al*., 2011).

Results detected no significant changes occur in SOD activity of TD treated healthy group and TD treated diseased group at zero, 3, 7, 14 and 28 days post-treatment compared to the control group. Also, other studies confirmed the same result after tilmicosin treatment in New Zealand rabbits (Altunok *et al*., 2002). In contrast, azithromycin and tilmicosin treatment decreased the level of SOD in rats and Bulb mice, while Tulathromycin increased ($P < 0.05$) the levels of serum SOD in rabbits (Yazar *et al*., 2002; Er *et al*., 2011; Atli *et al*., 2015).

CONCLUSION

P. multocida is one of the most prevalent causes of BRD in Egypt, and tildipirosin is highly effective as a prophylactic and metaphylactic treatment against BRD cases caused by *P. multocida*, and it has a potentially anti-inflammatory effect.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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