

Efficacy of tilmicosin for treatment of pasteurellosis in Holstein calves: A controlled clinical trial

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ABSTRACT

Objective: To assess the effectiveness of Tilmicosin in the treatment of pasteurellosis in Holstein calves.

Design: Controlled study.

Animals: Twenty Holstein Calves

Procedures: 10 Holstein Calves suspected to be suffering from Bovine respiratory distress (BRD) were treated with Tilmicosin and another 10 Holstein healthy calves under the same conditions of hygiene, nutrition, and management were used as a control group. The nasal swap was collected from the two groups for bacteriological isolation and blood samples were collected for hematological and biochemical analysis at zero, 7th, 14th days.

Results: Bacteriological isolation revealed that *Pasteurella multocida* and *Mannheimia haemolytica* serotype A were the most causative agents of pasteurellosis in the infected calves. The hematological analysis of the tilmicosin treated group showed a significant decrease ($P<0.05$) in RBCs at zero week and the second week, with a significant increase ($P<0.05$) in WBCs at the second week, with no significant change ($P>0.05$) in hemoglobin, but there is a significant decrease in hematocrit at Zero Week and second week, and a significant decrease ($P<0.05$) of MCV at zero week and first week, with a significant increase of MCH at zero weeks, with a significant increase in MCHC at zero weeks and the second week with a significant increase in Plt at second week, a significant decrease in lymphocyte at zero week and second week but there is a significant increase of neutrophil at zero weeks and the second week with a significant decrease at the first week. The biochemical analysis showed no significant change in total protein and albumin with a significant increase in GOT at the first week, a significant decrease in GPT at second week, a significant increase in ALP at Zero weeks, and first week there is a significant increase in Gb at zero weeks, first week and second week, albumin concentration was significantly decreased, a significant decrease of creatinine at zero weeks and a significant increase in the first week.

Conclusion and clinical relevance: In conclusion, tilmicosin induced a significant decrease in RBCs & lymphocyte and a significant increase in WBCs, neutrophil, platelets, and globulin.

Keywords: Tilmicosin; Pasterullosis; Calves

1. INTRODUCTION

Bovine respiratory disease (BRD) is a general term for respiratory disease in cattle and the most ubiquitous disease all over the world. Bovine respiratory disease is also one of the most major causes of morbidity and mortality with severe economic losses in the cattle industry due to loss of production, labor, increased time on feed, prophylaxis, and metaphylaxis treatments [1], [2].

They are often referred to as BRD is responsible for subvert the cow-calf business and the disease result from dramatic interaction between the stress condition (environmental conditions and herd management), causative agent (virus agents and bacterial agents), and animal immunity. These agents often produce mild clinical to severe clinical signs and death within 24 to 36 hours or may cause permanent lung

damage as fibrosis, adhesions, and/or abscesses, in chronic cases, which will impact performance. That is why early recognition and treatment of BRDC are so important [3], [4] & [5]. However, the affected calves exhibiting signs of fever, depression, off food, separate themselves from the rest of the pen, abnormal nasal discharge, cough, and abnormal lung sound [6-7].

Pasteurella is a major cause of severe "shipping fever" pneumonia when combined with stress and with and without viral agents. The major bacterial pathogen involved in pneumonic pasteurellosis of cattle is *Mannheimia haemolytica* serotype 1 [8].

Macrolides are active against important animal pathogens and their spectrum in general covers Gram-positive bacteria

and Gram-negative bacteria like *Mannheimia haemolytica* and *Pasteurella multocida* [9]. Macrolides also have significant immunomodulatory effects independent of their antimicrobial activity; for example, it has been shown to enhance the pro-inflammatory reaction of the host, to improve phagocytosis and to reduce local inflammation [10].

Tilmicosin is one from the macrolides group which used successfully as a prophylactic for infection in feedlot animals [11] and is also an effective therapy [12] for undifferentiated bovine respiratory disease. The most frequent bacterial pathogen encountered in these infections is *Mannheimia haemolytica* –A1 [13]. Young calves are also susceptible to infection with *Mannheimia haemolytica* as a secondary invader within the enzootic pneumonia complex [14].

The current study was conducted to evaluate the effectiveness of Tilmicosin for the treatment of Holstein calves suffering from clinical signs of BRD with special reference to some hematological and biochemical parameters.

2. MATERIALS AND METHODS

The experimental protocol was approved by the Faculty of Veterinary Medicine, Mansoura University, Egypt and Animal Health Research Institute Committee on Animal Care.

2.1. Animals

Our study was conducted in a Holstein dairy farm at Dakahlia Governorate suffered from a high incidence of BRD clinical signs especially in the calves (40:70 days age-old) with body weight ranged from 65 to 80kg. Calves that suspected to suffer from BRD were visually examined for the presence of nasal discharge, respiratory distress, cough, depression, and inappetence. After clinical signs observation, the rectal temperature of the calf was recorded using the clinical scoring system [15]. Ten diseased calves that suffered from BRD were chosen as a diseased group compared to another healthy ten calves has served as a control group. All tested animals were kept under the same conditions of hygiene, nutrition, and management. Clinical examination for all calves was done according to Radostits et al., [16].

2.2. Treatment

First diseased group injected by Tilmicosin (Pneumotac®: each ml contain Tilmicosin phosphate 333.828 mg equivalent to Tilmicosin base 300 mg, manufactured by ADWIA co. S.A.D. 10th Ramadan city, Egypt) in dose 1ml/ 30 kg B.W. S/C [17], while the second group was kept as a control group.

2.3. Samples

Nasal swabs from clinically diseased calves were taken for bacteriologically isolation and identification as described by Kabeta et al. [8]. Blood samples were collected at 0, 7 and 14 days after treatment and used for hematology and biochemical analysis. Two blood samples were collected from each calf

using a jugular vein puncture. The first blood sample was collected on a labeled test tube containing 5 mg K₂EDTA in a concentration of 1 mg/1ml blood [2] as an anticoagulant for the determination of hematological parameters (RBCs count, Hb content, and PCV %). The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3000 rpm for 10 min, and then the clear non-haemolyzed serum samples were separated and stored at –20°C until subsequent biochemical analysis.

2.4. Hematological and Biochemical analysis

Total erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), Total leukocytic count (WBCs), and differential leukocytic counts were determined by a hematological analyzer as previously described by Jain, [18]. Serum total proteins (TP) were determined spectrophotometrically according to the method described Pagana and Pagana [19]. Albumin was determined calorimetrically using the dye-binding technique with bromocresol green and the A/G ratio was calculated by dividing the albumin value over globulin value according to Fischbach and Dunning [20]. Serum globulin was determined by the differences between total protein and albumin according to Chernecky and Berger [21]. Serum samples were used also for the determination of aspartate transaminase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and lactate dehydrogenase (LDH) using the special kits according to the method described by Pagana and Pagana [19], Thefeld [22] and Ghanem et al. [23] respectively. Serum urea and creatinine concentrations were also determined spectrophotometrically using special kits according to the method described by Walker et al., [24], Peake and Whiting [25], respectively.

2.5. Bacteriological Isolation of *M. haemolytica* and *P. multocida*

Bacteriological Isolation of *M. haemolytica* and *P. multocida* in the current study was performed according to Rimler and Rhoades, [26]. The Media and reagents for biochemical tests were prepared according to Jamaludin et al. [27] while, Serotyping of *P. multocida* isolates were analyzed using rapid slide agglutination test using capsular type B antiserum according to Heddleston et al., [28] and Rimler and Rhoades, [26].

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 20 (IBM Armonk, NY, USA). The data were statistically analyzed using an independent sample *t*-test to compare healthy with the diseased animal as previously described by Graham et al., [29]. Values were represented as means ± standard error (SE). All differences were considered statistically significant when *P*<0.05.

3. RESULTS

3.1. Clinical examination of calves under our experiment

Clinical examination of animals under study showed a significant elevation ($P<0.05$) of body temperature of diseased calves. Moreover, a significant decrease ($P<0.05$) in ruminal movement rate was recorded in diseased animals compared to healthy calves. Nasal discharges with various degrees and ocular discharge were recorded in diseased animals compared to the control group. In details, two-calves showed mucoid discharge. While, mucopurulent and purulent discharges were observed in five and three- calves, respectively. Significant increases ($P<0.05$) in respiratory and coughing rates were observed on diseased calves (Table 1).

Table 1. Clinical findings calves with pasteurellosis.

Parameter	Control (n=10)	Diseased (n=10)
Body Temp °c	38.7± 0.31 ^b	40.81±0.23 ^a
Ruminal movement/minute	3.54± 0.21 ^a	1.87±0.14 ^{ba}
Respiratory rate/minute	35.86±2.18 ^b	60.54±3.54 ^a
Coughing /minute	0.12±0.01 ^b	8.65±0.23 ^a

Table 2. Biotyping of *Pasteurella multocida* and *Mannheimia haemolytica* isolates.

No. of isolates	<i>Pasteurella multocida</i> Capsular typing		
	A	B	C
3	2	1	0
No. of isolates	<i>Mannheimia haemolytica</i>		
	<i>M. haemolytica</i> A		<i>M. haemolytica</i> T
Susceptibility to penicillin	Sensitive		Resistant
Arabinose fermentation	+		-
Trehalose fermentation	-		+
No. of isolates	7		0

3.2. Bacteriological isolation

The obtained results showed that 30% of the nasal swap isolates from the infected calves was *Pasteurella multocida* and 70% was *Mannheimia haemolytica* serotype A.

3.3. Clinical score for Cure rate

Out of ten diseased animals, the animals exhibited loss depression, appetite loss, change in respiratory pattern, and fever were 3, 1, 4, and 2, respectively. Depend on the Dart method the cure rate for the calves under experiment which treated with tilmicosin 10mg /kg. BW. by s/c was 7.5%. After one week of the experiment, one of the treated calves has died, post mortem examination was applied on the carcass, and samples from the lung were sent for histopathological examination.

3.4. Hematological analysis

The comparison between tilmicosin 10mg/kg.bwt .s/c injected calves group and control group show a significant decrease in RBCs count in zero weeks and the second week for the infected group, and that is confirmed by the hematocrit blood test (HCT) and red blood cells distribution width (RDW-CV). The significant decrease in platelet count (PLT) in the second week for the tilmicosin-treated group with no significant change in mean platelet volume (MPV) and a significant decrease in platelet distribution width (PDW-CV) in zero weeks and the first week for tilmicosin treated group. (Table 3).

Table 3. Hematological parameters of control and Tilmicosin-treated groups by S/C injection in Holstein calves.

parameter	group	Zero week	First week	Second week
RBCs X10 ⁶	control	3.95±0.46 ^a	3.96±0.78 ^a	4.87±0.41 ^a
	Treated	2.78±0.64 ^b	4.46±0.88 ^a	3.98±0.15 ^b
WBCs X 10 ³	control	5.65±0.14 ^a	6.66±0.26 ^a	6.18±0.63 ^b
	Treated	5.58±0.49 ^a	5.85±0.35 ^a	8.43±0.44 ^a
Hgb (g/L)	control	6.70±0.53 ^a	6.91±0.48 ^a	6.73±0.57 ^a
	Treated	6.81±0.74 ^a	6.86±0.59 ^a	7.05±0.64 ^a
Hct (%)	control	16.01±1.13 ^a	14.81±2.95 ^a	16.45±1.70 ^a
	Treated	10.46±1.52 ^b	14.11±2.69 ^a	12.43±1.51 ^b
MCV	control	40.35±0.78 ^a	38.16±4.88 ^a	33.50±0.88 ^a
	Treated	37.28±0.49 ^b	33.00±1.71 ^b	34.66±2.49 ^a
MCH	control	17.31±0.82 ^b	15.05±1.47 ^a	13.86±0.29 ^a
	Treated	32.68±0.23 ^a	15.16±1.42 ^a	13.88±0.23 ^a
MCHC	control	43.46±2.86 ^b	40.56±1.53 ^a	41.46±0.99 ^b
	Treated	90.58±2.72 ^a	42.93±1.70 ^a	44.03±0.20 ^a
RDW-CV	control	16.18±0.78 ^a	11.05±1.56 ^a	15.20±0.51 ^a
	Treated	13.70±0.29 ^b	12.00±1.40 ^a	10.41±1.50 ^b
Plt X10 ³	control	660.66±48.66 ^a	2349.16±953.15 ^a	1488.00±185.29 ^b
	Treated	639.00±50.02 ^a	3110.33±811.76 ^a	3938.33±105.19 ^a
MPV	control	5.45±0.14 ^a	9.15±0.82 ^a	9.46±0.34 ^a
	Treated	5.80±0.24 ^a	9.96±0.35 ^a	10.61±0.32 ^a
Pct	control	0.35±0.03 ^b	0.03±0.03 ^a	0.29±0.01 ^a
	Treated	2.73±2.49 ^a	0.00±0.00 ^a	0.00±0.00 ^b
PDW-CV	control	15.15±0.22 ^a	3.05±1.93 ^a	2.98±0.18 ^a
	Treated	7.82±0.43 ^b	1.41±0.01 ^b	2.93±0.15 ^a
Lymphocyte (%)	control	64.00±4.38 ^a	53.33±5.71 ^a	70.00±5.18 ^a
	Treated	52.33±3.17 ^b	59.00±4.41 ^a	49.66±2.49 ^b
Monocyte (%)	control	4.66±0.33 ^a	4.00±0.36 ^a	4.50±0.50 ^a
	Treated	4.16±0.54 ^a	5.66±0.33 ^a	5.16±0.74 ^a
Neutrophil (%)	control	21.33±2.30 ^b	41.83±4.94 ^a	25.50±5.33 ^b
	Treated	41.83±2.45 ^a	35.33±4.31 ^b	45.16±2.49 ^a
Eosinophil (%)	control	0.00±0.00 ^a	0.50±0.00 ^a	0.00±0.00 ^a
	Treated	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

There is a significant increase in Procalcitonin (PCT) in zero weeks for the treated group with a significant decrease in the second week (Table 3), while there was a significant increase in WBCs in the second week for the tilmicosin treated.

3.5. Biochemical analysis

The changes in biochemical parameters for the tilmicosin 10 mg/kg.bwt subcutaneous injected calves showed a significant increase ($P<0.05$) in globulin level with on significant change ($P>0.05$) in TP and albumin level. However, the significant increase ($P<0.05$) in alkaline phosphatase level in the zero week and the first week for the same group indicates injury in the liver, gall bladder, and bone marrow but there was a significant decrease ($P<0.05$) of ALT liver enzyme in the second week (Table 4).. AST liver enzyme showed a significant increase ($P<0.05$) in the first week only (Table 4). In the present study, the renal function is reflected by urea level which showed a significant increase ($P<0.05$) in the first week (Table 4)

Table 4. Biochemical parameters of control and Tilmicosin (10 mg/kg b.wt) groups by S/C injection in Holstein calves.

Parameter	Group	Zero week	First week	Second week
Tp (g/dl)	control	6.18±0.51 ^a	7.05±0.36 ^a	5.58±0.54 ^a
	Treated	7.99±0.41 ^a	8.59±0.30 ^a	7.78±0.52 ^a
Alb (g/dl)	control	1.53±0.28 ^a	2.05±0.54 ^a	0.91±0.15 ^a
	Treated	1.77±0.29 ^a	2.23±0.49 ^a	1.08±0.07 ^a
AST (U/L)	control	34.50±3.27 ^a	25.33±0.98 ^b	30.16±2.54 ^a
	Treated	29.32±2.32 ^a	30.83±0.71 ^a	31.50±3.78 ^a
ALT (U/L)	control	10.33±0.89 ^a	7.93±0.44 ^a	8.00±0.84 ^a
	Treated	9.33±0.29 ^a	7.50±0.26 ^a	5.16±0.12 ^b
ALP (U/L)	control	9.56±0.13 ^b	9.57±0.60 ^b	12.63±1.38 ^a
	Treated	13.18±0.95 ^a	12.21±0.82 ^a	10.53±1.97 ^a
Urea (mg/dl)	control	41.91±2.05 ^a	29.39±2.18 ^a	35.45±2.72 ^a
	Treated	30.33±2.29 ^a	32.00±2.88 ^a	38.51±2.80 ^a
Creatinine (mg/dl)	control	1.23±0.20 ^a	0.65±0.03 ^b	1.07±0.12 ^a
	Treated	1.55±0.14 ^b	1.39±0.17 ^a	1.31±0.23 ^a
Globulin (g/dl)	control	4.65±0.34 ^b	5.00±0.52 ^b	4.67±0.51 ^b
	Treated	6.22±0.41 ^a	6.36±0.32 ^a	6.7±0.18 ^a

4. DISCUSSION

The signs of bovine respiratory disease were found among the infected animals that were found through the decrease in ruminal movement and increase in rectal temperature. Various degrees of ocular and nasal charges were also detected that was accompanied by a significant increase in respiratory movement and high coughing rates in diseased individuals. The infected pathogen detected in nasal swabs was mainly *P. multocida* (30%) as well as *M. haemolytica* (70%), which was found by a study conducted by Conlon et al., [30], where *M. haemolytica* showed the predominant isolate in stressed feeder calves.

The comparison between tilmicosin 10mg/kg.bwt .s/c injected calves group and control group show a significant decrease in RBCs count in zero weeks and the second week for the infected group, and that is confirmed by the hematocrit blood test (HCT) and red blood cells distribution width (RDW-CV) which show the same result to the infected group, also, to mean corpuscular volume (MCV) which show a significant decrease in zero weeks and the first week for the infected

group. And these results agree with Oztekin et al., [31] and Altunok et al., [32] who reported that tilmicosin caused significant changes in erythrocytic parameters in mice and rabbits. (Table 5).

Although there is no significant change in hemoglobin (Hgb) for the two groups, there is a significant increase in mean corpuscular hemoglobin in zero weeks, and mean corpuscular hemoglobin concentration in zero weeks and the second week for the tilmicosin treated group compared by the control group (Table 5) and these results are agreed with Scorneaux et.al. [33] and Altunok, et.al. [32] which did not record any change in Hgb after tilmicosin injection in the rabbit.

The significant decrease in platelet count (PLT) in the second week for the tilmicosin treated group with no significant change in mean platelet volume (MPV) and a significant decrease in platelet distribution width (PDW-CV) in zero week and the first week for tilmicosin treated group (Table 5). There is a significant increase in Procalcitonin (PCT) in zero weeks for the treated group with a significant decrease in the second week (Table 5).

The significant increase in WBCs in the second week for the tilmicosin treated group does not agree with Khan and Zafar, [34] which record there is a temporary decrease in WBCs with tilmicosin treatment, but the significant decrease in a lymphocyte in zero weeks and the second week for the tilmicosin treated group, a significant increase in neutrophil count in zero week and the second week for the same group with no significant change in monocyte and eosinophil. (Table 5), and agreed with the oxidative stress effect of tilmicosin in mice [32] and [35] and rats [36] and chicks [37].

The changes in biochemical parameters for the tilmicosin 10mg/kg.bwt.s/c injected calves show a significant increase in globulin level result from inflammatory effect with on significant change in total protein and albumin level. However, the significant increase in alkaline phosphatase level in the zero weeks and the first week for the same group indicate injury in the liver, gall bladder, and bone marrow but there is a significant decrease of ALT liver enzyme in the second week with a significant increase of AST liver enzyme in the first week only. The renal function is reflected by urea level which shows no significant change with a significant decrease level in zero weeks and a significant increase in the first week.

In conclusion, tilmicosin induced a significant decrease in RBCs & lymphocyte and a significant increase in WBCs, neutrophil, platelets, and globulin.

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