SYNERGISTIC EFFECT OF VOLATILE OILS AND LONG-ACTING OXYTETRACYCLINE IN TREATMENT OF RESPIRATORY DISEASES IN CALVES

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ABSTRACT

A field trial was conducted to evaluate the efficacy and safety of a mixture of volatile oils either alone or co-administered with long-acting oxytetracycline in treatment of undifferentiated bovine respiratory disease (BRD). Thirty eight newly born calves (2-5 month old) belonging to a private farm in Sharkia province, were used in this study. Five apparently healthy calves were kept as a control group. The remaining calves showed a different signs of bovine respiratory disease. After bacterial isolation and identification of the causative microorganisms, the calves were assigned to three equal groups (each of 11 calves). The first group (A) was treated intramuscularly with oxytetracycline 20% L.A. (20 mg/kg bodyweight), the second group (B) was treated with aeroforte volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days, while the third group(C) was given volatile oils with long-acting oxytetracycline. Blood and serum samples were collected from five calves of each group (infected with Pasteurella spp and staphylococcus aureus) just before treatment, three and ten day post-treatment for hematological and biochemical studies.

The bacteriological examination revealed that Pasteurella spp, Haemophilus spp and E.Coli were the most common isolates. The blood picture of the diseased calves, before treatment showed a significant decrease in the erythrocytic count, Hb concentration and PCV. Hypoalbuminemia and hyperglobulinemia with a significant elevation in total leukocytic counts, haptoglobin and C-reactive protein were recorded in diseased calves.

The present study revealed that administration of volatile oils with long-acting oxytetracycline was more effective in treatment of undifferentiated bovine respiratory disease (BRD). All the parameters retrieved to their control levels at the end of the experiment in all treated calves.

INTRODUCTION

Respiratory diseases (RD) are a major disorder in cattle world-wide and represent an important cause of economic losses (**Easlemont and Kossaibati, 1997**). It is generally known that Respiratory diseases in calves are of multifactorial aetiology. Environmental conditions and viral diseases as bovine respirato-

ry syncytial virus, parainfluenza virus 3 and bovine herpes virus may facilitate the invasion of bacteria such as Pasteurella multocida, Mannheimia haemolytica or Mycoplasma spp **(Caldow, 1996)**.

Several injectable antibacterials can be used to treat animals with RD such as tilmicosin, florfenicol and oxytetracycline **(Doherty et al., 2001)**.

Oxytetracycline is a broad-spectrum antibiotic which is active not only against bacteria but also against some chlamydia, rickettsia and protozoa. This drug is widely used in veterinary medicine due to its wide spectrum and also a number of advantageous pharmacokinetic features of the newer, long-acting formulations, in particular persistence of concentrations above the minimal inhibitory concentration (MIC) of 0.1-1.0 µg/ml for periods longer than the ordinary preparations **(Rossemblatt, 1980)**.

Nowadays the development of antibiotic resistance among microorganisms is a public health concern and this drives the search for new and more safely antimicrobial agents. The essential oils can constitute a powerful tool to reduce the development and dissemination of antimicrobial resistance **(Toroglu, 2011)**. Aeroforte is a natural product based on the essential oils (peppermint, eucalyptus, thyme, fir needle, lemon and menthol) which have a positive effect on animals suffering from heat stress and respiratory problems. It brings relief to cows with breathing problems due to respiratory tract diseases or cough.

Peppermint oil can be used as a remedy for respiratory problems such as nasal congestion, asthma, pneumonia, bronchitis, and dry coughs **(Admin, 2011)**.

Eucalyptus essential oil is effective for treating a number of respiratory problems including cold, cough, running nose, sore throat, asthma, nasal congestion, bronchitis and sinusitis. This oil is antibacterial, antifungal, anti inflammatory and decongestant which makes it a good ingredient in many medicines for treating respiratory problems **(Sadlon and Lamson, 2010)**.

The objective of this study is to evaluate the efficacy and safety of a mixture of volatile oils (peppermint, eucalyptus and menthol oils) either alone or co-administered with the long-acting oxytetracycline in treatment of undifferentiated bovine respiratory disease (BRD) during post- weaning period of the calves.

MATERIALS AND METHODS

Drugs :

1. Long- acting oxytetracycline (oxytetracycline 20%L.A.)® (Arab Company for Medical Products). It was given as a 20% solution: each ml contains 200 mg oxytetracycline. It was administered intramuscularly as a single dose of 20 mg/kg bodyweight **(Deleforge et al., 1994)**.

2. Volatile oils (aeroforte) ® (Kanters Company) :

It is a mixture of peppermint, eucalyptus, thyme, fir needle, lemon and menthol oils. The recommended dose is 2ml/10 liter of drinking water / 24 hours for 3 consecutive days

Animals :

Thirty eight newly born calves (2-5 month old) belonging to a private farm in Sharkia province, were used in this study. Five of these calves were apparently healthy and kept as a control group. The remaining thirty three calves were suffering from respiratory signs in the form of nasal discharge, cough, fever, congested mucous membrane, lacrimation, bronchial rales and abnormal lung sound. After bacterial isolation and identification of the causative microorganisms, the calves were assigned to three equal groups (each of 11 calves). The first group (A) was treated intramuscularly with a long-acting oxytetracycline formulation at a single dose of 20 mg/kg bodyweight, the second group (B) was treated with volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days while the third group (C) was treated with volatile oils co-administered with long-acting oxytetracycline.

The following clinical observations were recorded on daily basis in a form of clinical illness index score (CIIS): body temperature (°C), breathing (breaths/min) and heart rate (beat/min), nasal discharge (mucous, mucopurulent, purulent), soft coughing, dyspnea, appetite (anorexia), signs of depression and mortality **(Bednarek et al., 2003)**.

Bacteriological examination :

Sterilized swabs were taken from nasopharyngeal of apparently health and diseased calves before and after treatment. The samples were inoculated into nutrient broth and incubated at 37°C for 24 hours, then subcultured into selective media according to **Woldehiwet et al. (1990)**. All bacterial isolates were identified according to colonial morphology, microscopically by Gram's stain and biochemically **(Holt et al., 1994)**.

Blood samples :

Two blood samples were collected from five calves from each group (infected with Pasteurella spp and staphylococcus aureus) through jugular vein puncture just before treatment, 3 and10 day post treatment. The first one was taken on heparin as anticoagulant for hematological examination. The second blood sample was left to clot at room temperature for about 2 hours, stored overnight in a refrigerator at 4°C and centrifuged at 3000 rpm for 15 min. Serum samples were drawn in dry clean capped tubes and kept in deep freeze at 20°C for biochemical analysis.

Table 1. Clinical Illness Index Scores (CIIS) for Calves.

Score	Description	Appearance
1	Normal	No abnormalities noted
2	Slightly ill	Mild depression, gaunt, +/- cough
3	Moderately ill	Severe depression, labored breathing, ocular/nasal discharge, +/- cough
4	Severely ill	Moribund, near death, little response to human approach

After the course of treatment the calves were re-examined again by clinical examination.

Hematological study :

The erythrocytic count (RBCs), hemoglobin concentration (Hb %) and packed cell volume (PCV %) were determined. The erythrocytic indices "Mean corpuscular hemoglobin" (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), were performed mathematically. Moreover, the total leukocytic and differential counts were conducted according to **Coles (1986)**.

Proteinogram :

Total protein was measured according to **Peters (1968).** Electrophoretic analysis was carried out for determination serum albumin, alpha (α), beta (β) and gamma (γ) globulins according to the technique described by **Davis (1964)**.

Anti-inflammatory markers :

Serum haptoglobin level was determined by means of sodium dodecyl sulphat-polyacrylamide gel electrophoresis (SDS-PAGE) according to **Yoshino et al. (1992)**. Serum C-reactive protein was determined according to the methods reported by kits of Biosystems S.A. (Spain) & Bio-Med Diagnostics (Egypt).

Statistical analysis :

The obtained data were analyzed statistically using an ANOVA test according to **(SPSS, 2001)**.

RESULTS AND DISCUSSION

Essential oils, produced by plants, have been traditionally used for respiratory tract infections, and are used nowadays as ethical medicines for cold **(Federspil et al., 1997)**. The recent approach of using volatile oils and antibiotics in combination constitutes a strategy to overcome the problems of resistance and side effects associated with conventional antibiotics.

In the present study the bacteriological examination of the cultured swabs from the diseased calves revealed that the predominant bacterial pathogens were Pasteurella spp. 1233 / (36.4%), Haemophilus spp. 9 / 33 (27.3%), Escherichia coli 6/33 (18.1%), Staphylococcus aureus 3 / 33(9.1%) and Streptococcus pyogenes 3 / 33 (9.1%) as shown in Table (2). Our results are in agreement with those recorded by **Soheir and Emad (1990)** who isolated Pasteurella spp. at a percentage of 40% followed by Haemophilus spp.

The obtained data in this study revealed that, the isolated bacteria were highly sensitive to volatile oils co-administered with oxytetracycline. This co-administration was proved to be more effective than oxytetracycline or volatile oils alone in treatment of undifferentiated bovine respiratory disease in calves. **Musser et al. (1996)** found that long-acting oxytetracycline was successfully used in the treatment of calf pneumonia worldwide. It is true and fitting to mention that antibacterial activity of essential oils such as thyme, as well as its main component (thymol) could be related to their most abundant components **Amy Morris (2010) Tohidpour et al. (2010) and Horváth et al. (2011)**.

On the same ground, **Li et al. (2011)** reported that, peppermint oil was active against

S. aureus with minimal inhibitory concentrations (MICs) ranging from 64-256 µg/mL. Similarly, essential oils as eucalyptus oil and its major component, 1, 8-cineole, have antimicrobial effects against many bacteria, including Mycobacterium tuberculosis and methicillin-resistant Staphylococcus aureus (MRSA), viruses, and fungi (including Candida) **(Sadlon and Lamson, 2010, Sokovic et al., 2010 and Ben Marzoug et al., 2011)**.

Our obtained results added further support to those previously reported which suggested that peppermint oil and menthol displayed additive synergy with oxytetracycline **(Schelz et al., 2006)**. Another report demonstrated that the combination of the essential oils (Peppermint) with antibiotics can be used for protection against some bacteria and reduction of drug resistance. The synergistic effect could lead to novel choices for the treatment of infectious diseases **(Rosato et al., 2007; Malik et al., 2011 and Toroglu, 2011)**.

Treatment of diseased calves with volatile oils co-administered with long-acting oxytetracycline (Group C) was associated with a significantly faster ($P < 0.05$) improvement in CIIS (cough, nasal discharge, dyspnea, depression and anorexia), especially on the 3rd day of treatment (Fig. 1). Also on the 4th and 5th day of observation the improvement in CIIS was more pronounced in Group C, however, the differences in comparison to other groups, were not statistically significant. At the end of the observation, body temperature in all groups returned to normal, but breathing and heart rates as well as CIIS were still the highest in Group A, treated with oxytetracycline alone.

In the present work, diseased calves showed a significant decrease in RBCs count, Hb concentration, PCV and a non significant change of MCH and MCHC as shown in Table (3). Similar results were reported by **El-Bealawy (2003)** in calves suffering from pneumonia. These results may be attributed to sequestration of iron in bone marrow macrophages and hepatocytes during infection, thus became unavailable for utilization in hemoglobin synthesis, resulting in inhibition of erythropoiesis **(Coles 1986)**.

Leukocytosis including neutrophilia, monocytosis, eosinophilia and lymphocytopenia were observed in diseased calves indicating the presence of inflammation caused by bacterial infection **(Coles 1986)**. The previous findings were similar to those obtained by **Galhoom et al. (2002)**.

In the present investigation, it has been demonstrated that, treatment of diseased calves with oxytetracycline, volatile oils and volatile oils co-administered with oxytetracycline resulted in an improvement in blood picture which returned to its control level, 10th day post-treatment especially in calves treated with volatile oils co-administered with oxytetracycline.

The proteinogram of the diseased calves before treatment demonstrated that the total serum proteins were non significantly altered due to the increase of serum globulin in response to respiratory infection (Table, 4). Our results were supported by that recorded by

Khodary and Rizk (2000). The significant decrease of serum albumin in diseased calves could be attributed to the destructive effect of bacteria and its toxins on the liver cells which are the main sources of albumin and protein synthesis in the body **(McPherson, 1984)**.

Diseased calves disclosed significant increase in serum α-globulins level. The previous findings were subtly explained by **Butler (1983)** who highlighted the fact that, serum α-globulins level usually increases in response to bacterial infection due to increase the level of ceruloplasmin and haptoglobulin which are two of the main components of α-globulins, used as a marker for acute bacterial infection. Total β-globulin and γ-globulin showed higher values indicating the activation of the immune defense of calves due to infection **(Coles 1986)**.

The current work proved that, the treatment of diseased calves with volatile oils alone or co-administered with oxytetracycline revealed a significant increase in serum total protein, serum albumin concentration and serum globulin concentration with a significant decrease in α-globulins compared with the diseased calves.

Results of this study are consistent with the previous work which reported that essential oils supplementation (94 mg/calf/day) significantly ($p<0.05$) increased serum total protein concentration when compared with the control, however, higher dose of essential oils supplementation (187 or 281 mg/calf/day) non significantly ($p>0.05$) increased serum total protein concentration. Moreover, essential oils supplementation at different levels increased serum albumin concentration while serum globulin concentration decreased within the higher addition levels of essential oils. Essential oils supplementation at 94 or 187 mg/calf/day recorded the highest values of globulin concentration and that may be attributed to enhance the resistance of calves against different stress factors. Higher serum total protein with essential oils supplementation may be indicated that essential oils had positive immune stimulatory effect of the calves during pre-weaning period **(Soltan 2009)**.

In the present investigation, it has been demonstrated that, the diseased calves before treatment disclosed a significant increase in serum haptoglobin and C-reactive protein compared with health calves. The concentration decreased in all treated calves (Table, 5).

Results of this study are consistent with the previous findings of **Wittum et al. (1996) and Orro et al. (2011).** The authors reported that serum concentrations of haptoglobin in diseased calves increased significantly. The concentration changes of these acute phase proteins were often accompanied with respiratory disease conditions. The concentration rapidly decreased in calves that recovered quickly. Haptoglobin values were 10 to 100 times higher than in the normal calves.

On the same ground, **Rantzsch et al. (2009)** reported that, essential oils as eucalyptus oil had a potent anti-inflammatory effect. Furthermore, it has been found that thyme oil suppresses the COX2 enzyme through the same mechanism of the NSAID

(non-steroidal anti-inflammatory) medicine; ibuprofenthat **(Hotta et al. (2010) and Kathleen (2010)**.

It could be concluded that co-administration of volatile oils with long-acting oxytetracycline was more effective in treatment of undifferentiated bovine respiratory disease (BRD). This higher efficacy was mirrored as complete recovery of clinical signs, hematological and protenlogram profiles.

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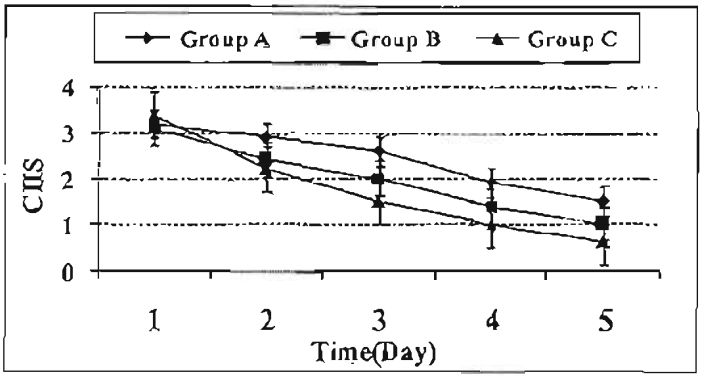

Fig. (1): Clinical illness index scores in calves suffering from respiratory disease and treated with oxytetracycline at a dose rate of 20 mg/kg bodyweight (A), volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days (B) and volatile oils co-administered with oxytetracycline (C). CIIS: cough (point 1), nasal discharge (mucous 0.5, mucopurulent 1, purulent 1.5), dyspnea (1), depression (1), anorexia (1). Significantly different in comparison to day 1 and other animal groups at $P < 0.05$

Table 2: Incidence of bacterial isolation from the nasopharyngeal swabs of diseased calves Pre-and Post- treated with long-acting oxytetracycline at a single dose of 20 mg/kg b. wt (A), volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days (B) and a combination of volatile oils with long-acting oxytetracycline(C) (Total number of examined calves=33)

	Incidence of isolation		A			B			C		
			Incidence of Isolation Pre-treatment	Incidence of Isolation Post-treatment		Incidence of Isolation Pre-treatment	Incidence of Isolation Post-treatment		Incidence of Isolation Pre-treatment	Incidence of Isolation Post-treatment	
	No.	%		No.	%		No.	%		No.	%
Pasteurella spp	12	36.4	4	1	25	4	3	75	4	-	-
Haemophilus spp	9	27.3	3	-	-	3	2	66.7	3	-	-
Staphylococcus aureus	3	9.1	1	-	-	1	-	-	1	-	-
Streptococcus pyogenes	3	9.1	1	-	-	1	-	-	1	-	-
Escherichia coli	6	18.1	2	1	50	2	1	50	2	-	-

Table 3: Hemogram of healthy and diseased calves before and after treatment with long-acting oxytetracycline at a single dose of 20 mg/kg b. wt. (A), volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days (B) and a combination of volatile oils with long-acting oxytetracycline(C) (n=5) (Mean±S.E)

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day post treatment			Diseased calves on 10th day post treatment		
			A	B	C	A	B	C
RBCs 10^6/UL	7.851±0.01^a	6.346±0.14^e	7.01±0.07^b	7.038±0.08^b	7.084±0.12^b	7.604±0.11^a	7.744±0.1^a	7.874±0.05^a
HB gm/dl	10.36±0.1^a	8.84±0.09^d	9.74±0.18^c	9.76±0.22^c	10.344±0.13^b	10.62±0.14^b	10.704±0.17^b	11.30±0.08^a
PCV %	36.38±0.1^a	29.32±0.09^e	32.66±0.55^b	32.06±0.73^b	33.08±0.31^b	35.26±0.4^a	35.58±0.51^a	36.32±0.19^a
MCV Fl	46.28±0.09^a	46.24±1.0^a	46.80±0.32^a	45.50±0.98^a	46.7±0.91^a	46.38±0.73^a	45.9±0.34^a	46.44±0.67^a
MCH Pg%	14.42±0.12^a	13.93±0.34^a	13.95±0.26^a	13.98±0.24^a	14.58±0.18^a	13.94±0.2^a	13.8±0.29^a	14.42±0.21^a
MCHC %	31.16±0.31^a	30.15±0.36^a	29.82±0.48^a	30.86±1.04^a	31.24±0.39^a	30.06±0.53^a	30.08±0.57^a	31.08±0.37^a
TLC 10^3/UL	9.526±0.08^d	12.32±0.04^a	11.702±0.09^b	11.42±0.11^b	10.358±0.09^c	9.664±0.21^d	9.598±0.23^d	9.408±0.03^d
Neutrophil 10^3/UL	4.352±0.017^d	7.582±0.014^a	6.538±0.07^b	6.302±0.11^b	5.282±0.12^c	4.70±0.17^d	4.454±0.19^d	4.34±0.09^d
Lymphocyte 10^3/UL	4.54±0.08^a	3.598±0.04^d	4.206±0.05^{b,c}	4.162±0.05^c	4.32±0.10^{a,b,c}	4.31±0.10^{a,b,c}	4.49±0.08^a	4.44±0.12^{a,b}
Eosinophil 10^3/UL	0.238±0.008^d	0.426±0.008^a	0.364±0.01^b	0.354±0.01^b	0.298±0.006^c	0.252±0.015^d	0.244±0.019^d	0.226±0.01^d
Monocytes 10^3/UL	0.396±0.009^d	0.714±0.009^a	0.594±0.01^b	0.608±0.01^b	0.456±0.02^c	0.40±0.01^d	0.41±0.02^d	0.404±0.01^d

Different letters in the same row means significant difference at ($p \leq 0.05$)

Table 4. Proteinogram of healthy and diseased calves before and after treatment with long-acting oxytetracycline at a dose rate of 20 mg/kg b. wt. (A), volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days (B) and a combination of volatile oils with long-acting oxytetracycline(C) (n=5) (Mean±S E)

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day Post-treatment			Diseased calves on 10th day post -treatment		
			A	B	C	A	B	C
Total protein (gm/dl)	7.18±0.04 d	7.212±0.01 d	7.284±0.02 d	7.72±0.08 b	7.37±0.05 c d	7.252±0.03 d	8.074±0.23 a	7.596±0.07 c b
Albumin (gm/dl)	3.868±0.02 c b	3.006±0.03 f	3.477±0.11 d	4.068±0.06 a b	3.678±0.13 d	3.74±0.05 c d	4.222±0.11 a	3.95±0.05 c b
Total α- globulin (gm/dl)	0.896±0.01 b	1.098±0.05 a	0.890±0.03 b	0.908±0.04 b	0.892±0.05 b	0.890±0.01 b	0.886±0.05 b	0.866±0.06 b
Total β – globulin (gm/dl)	0.922±0.01 c	1.062±0.02 a b	1.094±0.03 a	1.022±0.02 b	1.054±0.01 a b	0.892±0.01 c	1.02±0.03 b	1.03±0.02 a b
Total γ – globulin (gm/dl)	1.592±0.02 c	2.046±0.06 a	1.828±0.09 c b c	1.772±0.05 c b	1.746±0.07 c b	1.73±0.07 c b	1.946±0.11 a b	1.75±0.09 c b
Total globulin (gm/dl)	3.412±0.02 c	4.206±0.03 a	3.812±0.1 b	3.652±0.06 b c d	3.692±0.11 c b	3.512±0.06 d c	3.852±0.12 b	3.646±0.08 c d e

Different letters in the same row means significant difference at ($p \leq 0.05$)

Table 5. Mean values of serum haptoglobin and C-reactive protein in healthy and diseased calves before and after treatment with long-acting oxytetracycline at a dose rate of 20 mg/kg b. wt. (A), volatile oils at a dose rate of 2ml/ 10 liter of drinking water / 24 hours for 3 consecutive days (B) and a combination of volatile oils with long-acting oxytetracycline(C).

(n=5) (Mean±S E)

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day Post-treatment			Diseased calves on 10th day Post-treatment		
			A	B	C	A	B	C
Haptoglobin (gm/dl)	0.18±0.01 c d	1.06±0.05 a	0.31±0.02 b	0.31±0.02 b	0.24±0.03 c b	0.17±0.01 c d	0.17±0.01 c d	0.18±0.01 c d
C-reactive protein (gm/dl)	4.16±0.06 c	10.74±0.13 a	7.26±0.17 b	7.23±0.11 b	6.13±0.04 b	4.14±0.06 c	4.11±0.06 c	4.04±0.05 c

Different letters in the same row means significant difference at ($p \leq 0.05$)

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الملخص العربى

التأثير التآزرى للزيوت الطيارة والأوكسى تتراسيكلين طويل المفعول فى علاج أمراض الجهاز التنفسى فى العجول

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تعتبر الأمراض التنفسية من الأمراض الشائعة الحدوث فى العجول الصغيرة والتى تتسبب فى الكثير من الخسائر الاقتصادية لذلك تهدف هذه الدراسة إلى تقييم استخدام الإيروفورت (وهو عبارة عن مركب من الزيوت الطيارة) مع الأوكسى تتراسيكلين طويل المفعول (المضاد الحيوى الشائع الاستخدام) فى علاج الأمراض التنفسية التى تحدث نتيجة للإصابة البكتيرية ، وكذا دراسة تأثيرهما على بعض دلالات الإلتهاب ، وكذلك دراسة مدى الاستجابة المناعية بقياس البروتين الكلى والزلال والجلوبيلين (الفا-بيتا-جاما) وعمل صورة دم كاملة.

أجريت هذه الدراسة فى إحدى مزارع عجول التسمين الخاصة بمحافظة الشرقية على عدد ٣٨ عجل تتراوح أعمارهم بين ٢-٥ أشهر ويعانى ثلاثة وثلاثون منهم من أعراض تنفسية متمثلة فى إفرازات مخاطية من الأنف مع سعال حاد واحمرار الأغشية المخاطية وفقدان الشهية وارتفاع شديد فى درجة الحرارة. تم تقسيم العجول المصابة عشوائي إلى ثلاث مجموعات ، عولجت المجموعة الأولى (A) عضليا لمرة واحده بالأوكسي تتراسيكلين طويل المفعول بجرعة ٢٠ مجم/ كجم من وزن الجسم ، وعولجت المجموعة الثانية (B) بالزيوت المتطايرة بجرعة ٢ مللى/ ١٠ لتر من مياه الشرب / ٢٤ ساعة لمدة ٣ أيام متتالية بينما تم علاج المجموعة الثالثة (C) بالأوكسي تتراسيكلين طويل المفعول بالاشتراك مع الزيوت الطيارة. وعلى الجانب الآخر تم تخصيص عدد ٥ عجول سليمة ظاهريا وإكلينيكيا وغير معالجة وهى المجموعة الضابطة. تم متابعة العجول إكلينيكيا قبل وبعد العلاج لمدة ١٠ ايام لملاحظة الحالة الصحية والأعراض المصاحبة للمرض .كما تم جمع عينات دم من جميع العجول على مانع للتجلط قبل وبعد ٣ ، ١٠ أيام من انتهاء العلاج وذلك لفحص صورة الدم.كذلك استخدمت عينات السيرم لإجراء الفصل الكهربى لبروتين الدم والجلوبيولين وكذلك لقياس مضادات الألتهاب. وقد أظهرت النتائج ان الأمراض التنفسية تسببت فى العديد من التغيرات فى صورة الدم والعناصر البيوكيميائية التى تمت دراستها والتى انعكست سلبيا على الحالة العامة للعجول المصابة، وكشفت هذه الدراسة أن الأوكسي تتراسيكلين طويل المفعول بالاشتراك مع الزيوت الطيارة كان له أثر إيجابيا فى علاج العجول المصابة كما اتضحت كفاءة استخدام المضاد الحيوى مع الزيوت الطيارة فى شفاء جميع العجول المصابة وعودة جميع العناصر التى تم قياسها إلى معدلاتها الطبيعية.