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The Effect of *Thymus vulgaris* Volatile Oils in Controlling of the Complications Resulted from Spray of H120 Vaccine in Broiler Chicks

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Abstract: The most important genus of thyme is *Thymus vulgaris* known as a garden herb of thyme which its biologic and pharmacologic effects include; expectorant, antiseptic, anticough and also anti whooping cough. The recent studies show that thyme has significant effects too, on typhoid, diphtheria, colibacillus, pneumonic bacteria, staphylococcal, streptococcal bacteria and also has antifungal and antioxidant effects. In this research, 600 days old Lohman broilers in 5 groups of 30 selected as experimental and control groups and in all of these groups vaccination process and also determination of the final weight (FCR) have been used which in experimental group different doses of essence have been received and after vaccination the samples were taken and study process of hystopathological changes was done. Regarding to findings and statistical studies which were done SPSS software, the trachea edema factors, tracheitis, pulmonary edema, bronchitis have been surveyed. In the results obtained from the 6th day onwards IB vaccine (H120) of all above cases experimental group reduced by increasing dose.

Key words: *Thymus vulgaris* volatile oils, H120 vaccine, broiler chicks, antiseptic, anticough, Iran

INTRODUCTION

Avian Infectious Bronchitis (IB) was first described in the United States of America (USA) in the 1930s as an acute respiratory disease mainly of young chickens. A viral aetiology was established and the agent was termed avian Infectious Bronchitis Virus (IBV). The virus is a member of the genus Coronavirus, family Coronaviridae, in the order Nidovirales. IBV and other avian coronaviruses of turkeys and pheasants are classified as group 3 coronaviruses with mammalian coronaviruses comprising groups 1, 2 and 4 (group 4 being the more recently identified Severe Acute Respiratory Syndrome (SARS) coronavirus) (Cavanagh, 2003). Coronaviruses have a non-segmented, positive-sense, single-stranded RNA genome. IB affects chickens of all ages which apart from pheasants (Cavanagh *et al*., 2002) are the only species reported to be naturally affected. The disease is transmitted by the air-borne route, direct chicken to-chicken contact and indirectly through mechanical spread (contaminated poultry equipment or egg-packing materials, manure used as fertiliser, farm visits, etc.). IB occurs world-wide and assumes a variety of clinical forms, the principal one being respiratory disease that develops after infection of the respiratory tract tissues following inhalation or ingestion. Infection of the oviduct can lead to permanent damage in immature birds and in hens can lead to cessation of egg-laying or production of thin-walled and misshapen shells with loss of shell pigmentation. IB can be nephropathogenic causing acute nephritis, urolithiasis and mortality (Cavanagh and Naqi, 2003). After apparent recovery, chronic nephritis can produce death at a later time. IBV has also been reported to produce disease of the proventriculus (Yu *et al*., 2001). Vaccine and field strains of IBV may persist in the caecal tonsils of the intestinal tract and be excreted in faeces for weeks or longer in clinically normal chickens (Alexander and Gough, 1978; Cavanagh and Naqi, 2003). A detailed discussion of IBV antigen, genome and antibody detection assays prepared by De Witt (2000) is also available. IB vaccination programs in broilers involve the use of modified live vaccines. Vaccination of layers has historically involved administering a series of live vaccines and progressively increasing the aggressiveness of the route of vaccination (i.e., start with water administration and progress to fine particle spray) and strain of vaccine (highly attenuated to less attenuated). In breeders, a similar program is often followed however, prior to onset of production, an inactivated vaccine is also administered to stimulate antibody production. Inactivated vaccines stimulate higher levels of circulating antibodies than live vaccines and would be of value in a breeder program where maternal antibody protection is needed. However, modified live vaccines provide better

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stimulation of cell mediation (T-cell system) and elicit a superior local antibody (IgA) response as a result of local mucosal infection and thus would be of more value in protecting commercial layers. Thyme (*Thymus vulgaris* L.) is a perennial Labiatae of the Mediterranean region which has been used for centuries as spice, home remedy, drug, perfume and insectiside. In medicine, it is used as antispasmolytic, antibacterial, antifungal, secrotolytic, expectorant, antiseptic, anthelmintic and antitusive as reported by other researchers (Broucke *et al*., 1983; Broucke, 1983; Ozguven *et al*., 1987). A >50 plants are named and used as thyme in Turkey. Most of them belong to the genus Thymus but some of them to other genera of Lamiaceae such as Origanum, Majorana, Satureja and Thymbra (Mericli and Tanker, 1986). Thymol and carvacrol constituted the main phenolic compound of thyme oil. The major nonphenolic compounds were linalool and p-cymene (Piccaglia and Marotti, 1991). Thyme oil with high thymol content strongly inhibited the bacterial growth. Also, thymol has the higher activity against fungi followed by carvacrol and geraniol but linalool, terpineol and thujone exhibited the least effect (Broucke, 1983).

MATERIALS AND METHODS

In current study, about 600, 1 day old chickens, luhmann race, weighted 40±2.21 g was selected and was allocated into 5 groups (4 groups as treatment and 1 group as control). Each group was divided into 4 replication of 30 chicken. Nurturing condition was considered as same for all groups. Food and water was provided *ad libitum*. Food formulation was as follow; soya 380 kg, mineral supplements 2.5 kg, vitamin supplements 2.5 kg, D-calcium phosphate 15 kg, calcium carbonate 12 kg, salt 3 kg, methionine 2.5 kg, lysine 1.5 kg, corn 578.5 kg.

Vaccination: H120 vaccine was used as spray.

Extract administration: In for treatment groups, thyme extract was used with different doses. In control group, distilled water was used insisted of extract. Thymol density existed in extract was 25%. Used doses of thyme in treatment groups are shown in Table 1. Extract was administrated as drinking water from 1-7 days old as shown in Table 1.

Table 1: Used doses of thyme extract in treatment groups (ppm)

	Treatments			
Groups	1	2	3	4
Dose	100	200	250	300

Sampling: About 10 chickens from each group were selected and euthanized by electrical shock. Then, samples were obtained from upper respiratory tract and lungs and after fixation in formalin 10% was transferred to Histopathology Laboratory of Islamic Azad University, Veterinary Faculty, Tabriz Branch. In lab after processing and staining by H and E Method slides were prepared. Slides were assessed by light microscope.

RESULTS

Captured images are shown in Fig. 1-4. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 17.0 was used for statistical analysis. All data are presented as mean±SEM.

Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by

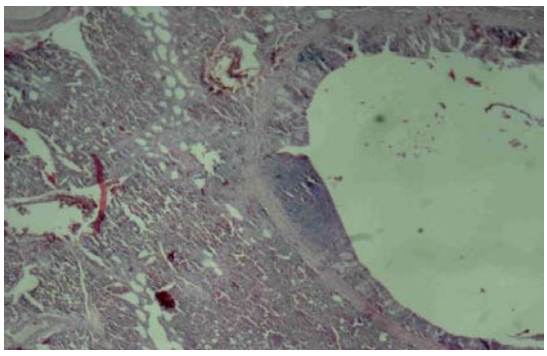

Fig. 1: Section of lung tissue of group 4 chickens. Lung is normal but there is a mild changes in secondary bronchiole (H and E, 10×)

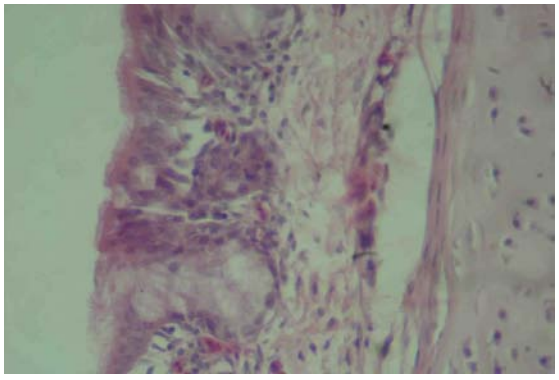

Fig. 2: Section of tracheal tissue of group 4 chickens. Mild tissue changes plus infiltration of mononuclear cells plus normal epithelium are obvious (H and E, 100×)

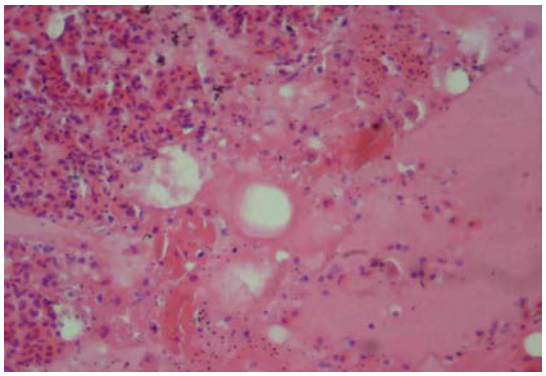

Fig. 3: Section of lung tissue of control group chickens. Severe hyperemia plus exude in the parabronchiole lumen are obvious (H and E, 100×)

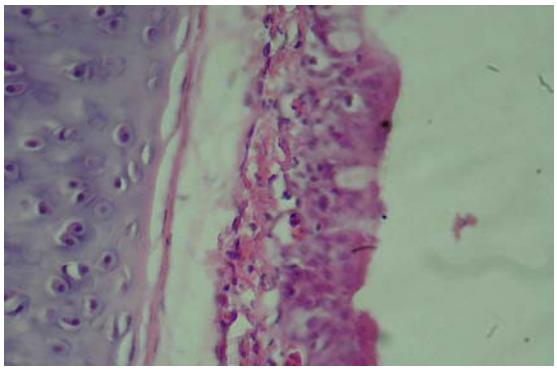

Fig. 4: Section of tracheal tissue of control group chickens. Losses of epithelial cilium plus infiltration of mononuclear cells are obvious. Also, edema in the sub-mucosal layer is shown (H and E, 100×)

Table 2: Comparative assessment of chick's performance on 7 days old

	Parameters		
Groups	Weight (g)	Food intake (g)	FCR
Treatment 1	128±2.11	110±0.11	1.25±0.01
Treatment 2	131±2.08	111±0.21	1.22±0.02
Treatment 3	129±2.10	113±0.07	1.26±0.01
Treatment 4	135±3.06	114±0.27	1.21±0.02
Control	125±2.05	108±0.15	1.28±0.02

Tukey's post-hoc multiple comparison test. $p<0.05$ was considered statistically significant. Based on results, there is a significant difference between groups from tracheal and pulmonary edema aspect ($p<0.001$). Based on results shown in Table 2 revealed that there is a significant difference between groups from weight aspect but there is not a significant difference between groups from FCR and food intake aspect.

DISCUSSION

Vaccines are used to prevent or reduce problems that can occur when a poultry flock is exposed to field disease organisms. Vaccinations should be thought of as insurance. Like insurance, there is a price to be paid for protection against a potential threat. Costs include price of the vaccine, time spent designing the vaccination schedule and administering the vaccines and losses due to vaccine reactions from the live-type vaccines and localized tissue damage from killed-type vaccine injections. As with insurance if the risk of a particular disease is low in the area, it makes little sense to vaccinate against that disease as the costs may outweigh the benefits.

Once a decision is made to vaccinate many factors have to be considered to ensure that vaccinations are successful. Before discussing the actual causes of vaccination failure, the two categories of vaccines commonly used in the commercial poultry industry must be described and the term vaccination failure must be defined.

A live-type poultry vaccine contains a virus or bacteria that must infect the chicken and multiply in its body to produce immunity, preferably with minimal reaction.

Multiplication of the virus in the chicken is necessary as only relatively small amounts of virus are administered to the bird. By multiplying in the chicken, increased amounts of virus are recognized by the chicken's immune system thus, an enhanced immune response results.

In one study that carried out by Mayor revealed that after administration of respiratory vaccines, mild signs resulted from vaccination reactions is determined. This finding is compatible with the research results that in control group same evidences was obvious but in treatment groups with increasing the doses these reactions was decreased that is consistent with Gruenwald *et al*. (2005) research results.

In one other study by Butcher after administration of the bronchitis vaccine, mild edema in the trachea, sever edema in submucosal layer and congestion and cells infiltration in the lamina propria with epithelial hyperplasia was seen.

CONCLUSION

These finding are compatible with the study results. In one study by Feizi and Nazeri (2011), revealed the *Thymus vulgaris* essential oils reduces vaccination reaction resulted from Newcastle disease.

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