# Some Biochemical, Haematological, Virological And Immunological Studies On Buffaloes Infected With Foot And Mouth Disease Virus

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## ABSTRACT

From the FMD outbreak in January (2006) in Kaleubia Governnorate (Egypt), twenty-five. dairy buffaloes were randomly chosen from buffaloes of private farms showed the symptoms of FMD, the animals were divided into five equally groups. The 1st group of buffaloes not FMD infected, not vaccinated, and served as the normal (negative) control group. The 2nd, 3rd, 4th and 5th groups were showed clinical signs of FMD, and previously vaccinated with FMD-vaccine (serotype-O), where the 2nd group not drug treated and served as the positive control group, the 3rd group was I/M. injected with 3mg/kg.b.wt. of oxytetracycline antibiotic, the 4th group was I/M. injected with 10mg/kg.b.wt. of enrofloxacin antibacterial and 5th group was I/M. injected with 2.5 mg/kg.b.wt. of levamisol anthelmentic. All drugs injected once a day, for 5-consecutive days, directly before sampling. At the 6th day post drug treatments, samples from pharynx, vesicles, ulcers and saliva from oropharyngeal cavity were taken for virological examination. Blood samples were taken for haematological investigation, serum samples were taken for serological, immunoelectrophoretical and biochemical investigations. Results indicated that the virus of FMD (serotype-O) could be isolated from the infected buffaloes inspite of their previous vaccination with FMD-vaccine (serotype O) (9-months ago). The drug treatments of FMD infected buffaloes indicated that the enrofloxacin showed strong immunosuppressive effect, so that it should be contraindicated in FMD infected buffaloes. In contrast, the levamisol drug showed strong immunostimulant effect to FMD infected buffaloes, so that it could be injected in FMD-infected buffaloes to reduce the severity and the coarse of the disease, and this drug may also be tried with FMD-vaccinated buffaloes to prolonging the time of high titres. However, the oxytetracycline showed weak immunosuppressive effect in diseased animals, so it could be suggested that oxytetracycline may injected by dose less than the therapeutic one from the 1st day of infection to combat the 2ry bacterial complications (with least side effects). We could also suggesting an experimental trials for periodically using polyvalent attenuated FMD virus vaccine prepared from local strains with eradication of diseased animals, in order to obtaining higher and longer antibody titres against FMD along with periodical levamisol treatment.

## INTRODUCTION

Foot and mouth disease (FMD) is one of the most economically important contagious diseases of the cloven hoofed animals *(1)*. The disease characterized by rapid spreading among herds and its ability to change its antigenic identity *(2)*. The clinical signs of the FMD in buffaloes are salivation, lamness, fever and presence of vesicles in and around mouth and on the feet. The disease has high morbidity and low mortality rates in adults, but has high mortality rates in young calves, it induced loss of weight and milk yield, and the secondary bacterial infections usually followed *(3)*.

The FMD virus (FMDV) belongs to family Picorna Viridae, genus Aphthovirus *(4)*. FMDV exists in seven immunologically distinct serotypes *(5)*, its genome is on 8.5 kilobase single strand RNA*(2)*. The virion consists of four proteins ($VP_1 \rightarrow VP_4$) of which the $VP_1$ is the main protein determining the antigenic identity of FMDV *(3)*.

In India, an outbreak of FMD between (1987-1991) was occur, the mortality rates in male buffaloes is higher than that in females (under the adult ages), and the deaths were enhanced by presence of other diseases associated with FMD, such as enteritis, hepatitis, digestive disorders or respiratory diseases, and the commonest metabolic disorders occomponied FMD were ketosis, rickets, hypovitaminosis-A and hypoproteinemia *(6)*. The buffaloes became infected and excrete virus before the clinical

signs of FMD developed. There were tongue lesions in cattle than in buffaloes. The foot lesions in buffaloes showed scaly appearance at first, but later become vesicular *(7)*, but the FMDV persisted longer with higher titre in buffaloes than in cattle *(8)*, so that , the buffaloes considered a true reservoir for infections with a number of FMDV-strains, and while the strains have been modified in the buffaloes, the pathogenicity in cattle remain prominent than in buffaloes *(9)*. The FMD-viraemia was persisted for up to 5 days, and the virus was isolated from the nose for up to 15 days *(10)*, but in carrier cases in African buffaloes, the virus could be isolated from some buffaloes up to 167 days post infection *(11)* depending on the duration of infectivity and the size of the available host *(10)*.

The currently available FMD-vaccines do not necessarily protect against the infection as opposed to disease condition. The available inactivated FMD-vaccines also only provide a transient immunity and need to be administered repeatedly to maintain high level of herd immunity *(12)*.

In Egypt, by the year 1998, an outbreak of FMD occurred in vaccinated and unvaccinated buffaloes and cattle, where in the tested animals it could isolated: serotype-O in 64.3% of the tested animals *(13)*. Recently in Egypt also, by the year 2006, an outbreak of FMD (serotype-A) was recorded in Ismaellia and Domyat Governorates *(14)*, and the FMD (serotype-O) could be also detected in Ismaellia Governorate in the same year 2006 *(15)*.

In Kaleubia Governorate (Egypt) an outbreak with typical clinical signs of FMD was detected in January (2006) in the dairy buffaloes which previously vaccinated with FMD-vaccine (serotype-$O_1$). The practitioners mostly used oxytetracycline antibiotic to combat secondary bacterial complications followed FMD infection, others used enrofloxacin antibacterial drug, so we used the two above drugs in the present study in two groups of infected buffaloes, we add the anthelmentic drug levamisol which used in a third group aiming to recognizing the immunodepressant or immunostimulant effects of such treatments, and also for understanding some biochemical and haematological changes associated with FMD viral infections in Egyptian buffaloes.

## MATERIAL AND METHODS

### (A) Vaccine

FMD-vaccine was obtained from veterinary serum and vaccine Research Institute (VSVRI), Abassia, Cairo. Each buffalo was S/C vaccinated with 2ml of inactivated FMD vaccine serotype-$O_1$ (9-months before the appearance of the infection).

### (B) Animals

Twenty dairy buffaloes were randomly chosen from infected herd of a private farm in Kaleubia Governorate with symptoms of FMD, all infected buffaloes were previously vaccinatede (9-month ago) with FMD-vaccine (serotype $-O_1$), another five non infected and non vaccinated buffaloes were obtained from a neighbouring farm, belonged to the same owner (with the same living conditions) to be used as a normal (negative) control animals.

### (C) Grouping & treatments

The twenty-five dairy buffaloes were grouped into five equal groups, the 1st. group was served as the normal (negative) control (non-vaccinated, not-infected, non-drug treated), the 2nd group was FMD-vacccinated, infected and drug not-treated, and served as the positive control group, the other groups were also vaccinated and infected, where the 3rd group was I/M injected, with 3 mg/kg.b.w of oxytetracycline (pan-terramycin, Pfizer, Co.). The 4th group was I/M injected with 10 mg/kg.b.w. of the antibacterial enrofloxacin (Uvetril, Uvedco Co., Jordan) and the 5th group was I/M. injected with 2.5 mg/kg.b.w. of the anthelmentic drug "levamisol" (Ucimisol, Amon Co.). All the drug were injected once a day, for 5-consecutive days directly before samplings.

### (D) Samples for virus isolation

Pharyngeal fluid and saliva samples were taken from the infected animals that showing clinical signs of FMD. Samples were

collected from vesicles and ulcers of mouth and from interdigital lesions of animals showing lamness. The pharyngeal fluid samples were collected using probang cups as described by Hedger and Stubbing *(16).* Primary isolation of FMD virus was carried out on monolayer cell-culture of bovine kidney cells. The isolated virus was typed using ELIZA typing kit provided by the FMD-World Reference Laboratory (WRL-pirbright, London, UK). The indirect Sandwich ELIZA was used according to Roeder and Smith (17), with a slight modification *(18).*

**(E) Blood and serum samples**

**(i) Blood samples:** The blood samples were taken from all buffaloes of all groups with EDTA-Anticoagulant for different haematological investigations as Red and White blood corpuscles and platelets counts, differential leucocytic counts, haemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the corpuscular haemoglobin concentration (MCHC) according to Schalm *(19).*

**(ii) The serum samples:** Serum samples were prepared from buffaloes of the 5-groups, for the following investigations :

**(1) Serological examination**

The Micro-Neutralization test according to (18) based on the method described by ***Golding et al. (20)*** using BHK-21 monolayer cells and the FMD virus isolates (A and O-serotypes). The titres of the test were expressed as $Log_{10}$-$TCID_{50}$.

**2. Determination of serum protein profile**

The serum protein fractions were determined through the serum protein immunoelectrophoresis using the cellulose acetate method (21).

**(3) Determination of some serum biochemical constituents**

Some serum biochemical constituents were determined as : Alanine aminotransferase (ALT) enzyme activity *(22),* and the concentrations of total bilirubin *(23),* total cholesterol *(24),* total protein *(25)* and the serum creatinine*(26).*

**(4) Statistical Analysis**

All the obtained data were statistically analyzed through the Analysis Of Variance (ANOVA) using F-Test, in order to determine the Least Significant Differences (LSD) between means, at $P \leq 0.05$, according to ***Snedecor Cochran (27).***

## RESULTS

**A. Clinical Signs**

The symptoms appeared in infected buffaloes as: fever, drop of milk yield, salivation, lameness, vesicles and erosions on the buccal mucosa, including the dental pads and interdigital space .

**B. Virus isolates and serotype**

The isolated causative agent is the foot and mouth disease virus (FMD-V) and its serotype is "O".

**C. Serum protein Electrophoresis**

***1. Albumin***

Only buffaloes treated with oxytetracycline showed significant increased in albumin than either infected non-treated positive control or normal (negative) control group of buffaloes.

***2. Alpha ($\alpha$) Globulin fraction***

The $\alpha$-globulins was significantly increased in non-treated (+ve control), oxytetracycline and levamisol treated groups than that of normal (negative) control buffaloes.

***3. The Beta ($\beta$) Globulin fraction***

The $\beta$-fraction was significantly increased in positive control group. The groups treated either with enrofloxacin or levamisol drugs induced increase of the $\beta$-fraction than that of the negative or positive control groups.

***4. Gamma ($\gamma$) Globulins fractions***

All infected groups of buffaloes (drug treated and non treated) showed significant increase of $\gamma$- globulins than that of negative (non-infected, non-treated) control, except in the oxytetracyclin treated group where the increase was non-significant. But when comparing with the infected non-treated (+ve

control) group, the enrofloxacin treated group showed significant decrease in $\gamma$-fraction. Oppositely, the levamisol treated group showed significant increase of the gamma-fraction than that of the all other groups.

*5. Albumin/Globulin (A/G) ratios*

No significant difference in A/G ratio between the positive and negative normal control group buffaloes. Only oxytetracycline treated group showed significant increase of A/G ratio than that of normal control group. Compared to the +ve control group, the oxytetrcycline treated group showed significant increase of the A/G ratio, but levamisol treated group showed significant decrease. The different immunoelectrophoretically protein fractions were tabulated in table (1)

**D. Haematological studies**

*1. White and Red Blood Corpuscles (WBCs and RBCs) counts*

All infected groups of buffaloes showed significant increase of WBCs and RBCs-counts compared with the normal control buffaloes. Compared to the infected, non-treated (+ve control) group, the oxytetracycline and enrofloxacin treated groups showed significant decrease in WBCs and RBCs counts, but the levamisol treated groups showed significant increase in both WBCs and RBCs counts.

*2. Haemoglobin (Hb) concentration*

The infected non-treated control group (+ve control) showed significant increase in Hb concentration than that of normal (non-infected, non treated) control group. Compared to positive control group (infected, non-treated), the Hb concentration was significantly decreased in oxytetracycline and enrofloxacin treated group, oppositely, the levamisol treated group showed significant increase in Hb concentration, than +ve control group.

*3. Packed Cell Volume (PCV)*

All FMD-infected groups of buffaloes (drug treated & non treated) showed significant decrease in PCV than that of the non-infected, non-treated negative control group.

*4. Mean Corpuscular Volume (MCV)*

All FMD-infected groups (drug treated and non-treated) showed significant decrease of MCV than that of the negative normal control group.

*5. Mean Corpuscular Haemoglobin (MCH)*

There was no significant change of MCH between buffaloes of +ve and –ve control groups. Compared to the +ve control group, the enrofloxacin treated group showed significant decrease of MCH.

*6.Mean Corpuscular Haemoglobin Concentration (MCHC)*

The MCHC was increased in all FMD infected groups than that of non-infected negative control group. Compared to FMD-infected positive control group, the oxytetracycline and enrofloxacin treated groups showed significant decrease in MCHC, but the levamisol treated group showed non-significant change than +ve control group.

*7. Blood platelets*

The platelets significantly decrease in positive control and in oxytetracycline treated groups than that of the normal negative control group, but it significantly increased in either enroflaxacin or levamisol treated groups than either positive or negative control groups (Table, 2).

**(E) Differential-Leucocytic Counts**

*1. Neutrophils*

The infected non-treated positive control group, the oxytetracycline and levamisol treated group showed significant decrease of Neutrophil percentages than that of non-infected normal control group. Enrofloxacin treated group not showed significant change than that of normal control group.

*2. Lymphocytes*

Compared to the normal non-infected and non-treated negative control group, the all infected non-treated (+ve control) and the all treated (except Enrofloxacin group) showed significant increase of lymphocyte percentages than normal control. Compared to the infected non-treated positive control group, the

oxytetracycline and levamisol treated groups showed significant increase of lymphocyte percentage than positive control, the levamisol treated group showed the highest and significant increase of lymphocyte percentage than all other groups.

*3. Monocytes*

Monocytes percentages not showed any significant changes between all groups of buffaloes.

*4. Eosinophils:*

No significant change of eosinophil percentage between all groups (except in enrofloxacia treated group, where it significantly decreased compared to either positive or negative normal control groups).

*5. Basophils*

Only, the infected, non-treated positive control group and the oxytetracycline treated groups were showed significant increase of Basophil percentages than that of the normal control group, oppositely, the enrofloxacin treated group showed significant decrease of Basophil percent than that of normal control group, but the lymphocyte percent of levamisol treated group not showed any significant change than that of the normal control group (Table, 3).

**(F) The Specific Antibody titres Against O-serotype of FMD-virus infection**

All FMD-infected groups of buffaloes showed significant increase of the antibody titre against O-serotype viral infection than that of the negative control group, and the highest titre showed by levamisol treated group. Compared to the positive control group (infected, non-treated), either oxytetracycline or enrofloxacin-treated groups showed significant decrease of the antibody titre than +ve control group, but the levamisol treated group showed significant increase of titre than that of the +ve control group (Table, 4).

**G. Serum Biochemical Changes**

*1. Alanine aminotransferase (ALT) Enzyme Activity*

FMD infection (drug and non-drug treated groups) was induced significant increase of ALT enzyme activity than that of non-infected non-treated, normal (negative) control groups. Compared to infected non-treated positive control group, all treatments induced significant increase of ALT-enzyme activity than that of positive control group. The highest ALT activity was showed by levamisol group.

*2. Total Bilirubin Concentraion*

FMD infection induced significant elevation of total bilirubin concentration than that of non infected normal control, all drug treated groups induced significant elevation of total bilirubin compared to infected non-treated positive control group. The highest bilirubin concentration was showed by oxytetracycline treated group.

*3. Serum Cholesterol Concentration*

All FMD infected groups (drug and non-drug-treated groups) were induced elevation of serum cholesterol concentration (except by levamisol treated group, where the elevation was non significant). The highest concentration was recorded by oxytetracycline treated group.

*4. Serum Creatinine*

FMD infection (in drug and non-drug treated groups) were induced significant elevation in serum creatinine concentration than that of normal (negative) control group. All drug treated groups significantly elevated the creatinine concentration compared to the infected non-treated (positive) control group (except by the levamisol treated group where the increase was non-significantly).

*5. Serum Total Protein*

*the* FMD infected non-treated (+ve control) group not significantly increase the serum total protein, but the infection with the drug treatments with oxytetracycline and levamisol could induced significant elevation of serum total protein than either negative (normal) control or positive (infected) control (Table, 5).

**Table 1. Serum Protein fractions (separated by electrophoresis) of the Different Groups of FMD infected Buffaloes**

| Groups | Albumin (g/dl.) | Alpha (α) Globulins (g/dl.) | Beta (β) Globulins (g/dl.) | Gamma (γ) Globulins (g/dl.) | Albumin/Globulin (A/G) ratios | Total protein (g/dl.) |
|---|---|---|---|---|---|---|
| Negative (normal) control | $3.400^{A}$ ± 0.369 | $0.710^{A}$ ± 0.074 | $0.610^{A}$ ± 0.012 | $0.980^{AD}$ ± 0.132 | $0.596^{ACD}$ ± 0.0.86 | $5.700^{AC}$ ± 0.316 |
| Positive control (infected) | $2.880^{A}$ ± 0.226 | $0.910^{B}$ ± 0.111 | $0.880^{B}$ ± 0.027 | $1.730^{BC}$ ± 0.211 | $0.811^{AC}$ ± 0.109 | $6.400^{AC}$ ± 0.127 |
| Oxytetracycline treated (infected) | $4.510^{B}$ ± 0.236 | $1.140^{C}$ ± 0.075 | $0.620^{A}$ ± 0.049 | $1.630^{CD}$ ± 0.086 | $1.340^{B}$ ± 0.107 | $7.900^{B}$ ± 0.211 |
| Enroflaxacin-treated (infected) | $3.000^{A}$ ± 0.191 | $0.840^{AB}$ ± 0.075 | $1.750^{C}$ ± 0.039 | $1.310^{D}$ ± 0.152 | $0.770^{C}$ ± 0.035 | $6.900^{C}$ ± 0.245 |
| Levamisol treated (infected) | $2.900^{A}$ ± 0.073 | $1.000^{BC}$ ± 0.102 | $2.088^{D}$ ± 0.125 | $2.520^{E}$ ± 0.304 | $0.520^{D}$ ± 0.026 | $8.500^{D}$ ± 0.196 |
| LSD (at $P \leq 0.05$) | 0.818 | 0.169 | 0.150 | 0.341 | 0.241 | 0.241 |

**N.B.** * The different letters in columns point to the significant change between means (at $p \leq 0.05$), * LSD = Least Significant Difference between means (at $p \leq 0.05$).

**Table 2. Haematological Parameters of the Different Groups of FMD infected Buffaloes.**

| Groups | WBCs ($x10^3/\mu l$) | RBCs ($x10^6/\mu l$) | Hb conc. (g/dl) | PCV (%) | MCV (fl) | MCH (pg.) | MCHC (g/dl) | Platelets ($x10^5/\mu l$) |
|---|---|---|---|---|---|---|---|---|
| Negative (normal) control | $3.50^{A}$ ± 0.141 | $8.10^{A}$ ± 0.127 | $15.20^{A}$ ± 0.179 | $60.50^{A}$ ± 3.162 | $74.70^{A}$ ± 2.04 | $18.80^{A}$ ± 0.894 | $25.100^{A}$ ± 1.649 | $220.00^{A}$ ± 3.162 |
| Positive control (infected) | $8.10^{B}$ ± 0.181 | $8.60^{B}$ ± 0.089 | $15.92^{B}$ ± 0.197 | $50.00^{BD}$ ± 3.688 | $58.00^{B}$ ± 2.154 | $18.00^{ABD}$ ± 0.860 | $32.00^{B}$ ± 1.897 | $193.00^{B}$ ± 2.884 |
| Oxytetracycline treated (infected) | $7.70^{C}$ ± 0.127 | $6.58^{C}$ ± 0.126 | $11.20^{C}$ ± 0.204 | $38.00^{C}$ ± 2.28 | $58.00^{B}$ ± 1.265 | $17.00^{BC}$ ± 1.019 | $29.00^{C}$ ± 1.265 | $100.00^{C}$ ± 4.472 |
| Enrofloxacin treated (infected) | $6.84^{D}$ ± 0.242 | $7.69^{D}$ ± 0.089 | $12.40^{D}$ ± 0.263 | $43.00^{CD}$ ± 2.154 | $56.00^{BC}$ ± 2.786 | $16\pm^{C}$ ± 1.414 | $29.00^{C}$ ± 1.442 | $334.00^{D}$ ± 4.308 |
| Levamisol treated (infected) | $8.40^{E}$ ± 0.152 | $9.20^{E}$ ± 0.179 | $15.90^{B}$ ± 0.228 | $49.00^{D}$ ± 3.059 | $53.00^{C}$ ± 2.154 | $17.00^{DC}$ ± 1.166 | $32.00^{B}$ ± 1.265 | $325.00^{E}$ ± 7.616 |
| LSD (at $P \leq 0.05$) | 0.215 | 0.201 | 0.453 | 7.531 | 3.173 | 1.709 | 2.244 | 11.898 |

**N.B.** * The different letters in columns point to the significant change between means (at $p \leq 0.05$), * LSD = Least Significant Difference between means (at $p \leq 0.05$).

**Table 3. Differential Leucocytic Count of the Different Groups of FMD infected Buffaloes.**

| Groups | Neutrophils (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) |
|---|---|---|---|---|---|
| Negative (normal) control | $45.00^{AD}$ ± 1.523 | $48.00^{AD}$ ± 2.280 | $3.00^{A}$ ± 0.283 | $2.00^{A}$ ± 0.282 | $2.00^{A}$ ± 0.400 |
| Positive control (infected) | $40.00^{B}$ ± 2.608 | $54.00^{B}$ ± 1.442 | $4.00^{A}$ ± 0.633 | $2.00^{A}$ ± 0.400 | $0.300^{B}$ ± 0.180 |
| Oxytetracycline treated (infected) | $34.00^{C}$ ± 1.442 | $61.00^{C}$ ± 1.811 | $3.00^{A}$ ± 0.849 | $2.00^{A}$ ± 0.632 | $0.300^{B}$ ± 0.18 |
| Enrofloxacin treated (infected) | $47.00^{D}$ ± 2.530 | $49.00^{D}$ ± 2.059 | $3.00^{B}$ ± 0.632 | $1.00^{B}$ ± 0.283 | $1.400^{C}$ 0.220 |
| Levamisol treated (infected) | $28.00^{E}$ ± 1.897 | $67.00^{E}$ ± 0.894 | $3.00^{A}$ ± 0.632 | $2.00^{A}$ ± 0.400 | $2.00^{A}$ ± 0.400 |
| LSD (at $P \leq 0.05$) | 4.788 | 3.072 | 0.793 | 0.793 | 0.600 |

**N.B.** * The different letters in columns point to the significant change between means (at $p \leq 0.05$), * LSD = Least Significant Difference between means (at $p \leq 0.05$).

**Table 4. The Serological Determination of the Specific Antibody Titres of FMD infected (serotype-0) Buffaloes of The Different Groups as Determined by the serum Neutralization Test.**

| Groups | FMD antibody titres against (0 –strain) ($Log_{10}$ – values) | Titre range |
|---|---|---|
| Negative (normal) control | $0.452^{A}$ ± 0.060 | ½ - ¼ |
| Positive control (infected) | $1.530^{B}$ ± 0.011 | 1/16 - 1/64 |
| Oxytetracycline treated (infected) | $1.350^{C}$ ± 0.063 | 1/16 - 1/32 |
| Enrofloxacin treated (infected) | $1.110^{D}$ ± 0.033 | 1/8 – 1/32 |
| Levamisol treated (infected) | $1.650^{B}$ ± 0.063 | 1/32 – 1/128 |
| LSD (at $P \leq 0.05$) | 0.145 | - |

**N.B.** * The different letters in columns point to the significant change between means (at $p \leq 0.05$), * LSD = Least Significant Difference between means (at $p \leq 0.05$).

**Table 5. Some Biochemical Constituents of the Different Groups of FMD infected Buffaloes.**

| Groups | ALT-Enzyme-Activity (U/L) | Total Bilirabin Conc. (mg/dl) | Cholesterol (mg/dl) | Creatinine (mg/dl) | Total Protein (mg/dl) |
|---|---|---|---|---|---|
| Negative (normal) control | $7.00^{A}$ ± 0.632 | $0.450^{A}$ ± 1.0220 | $98.00^{A}$ ± 2.280 | $0.850^{A}$ ± 0.051 | $5.700^{AC}$ ± 0.316 |
| Positive control (infected) | $9.00^{B}$ ± 1.020 | $0.540^{B}$ ± 0.020 | $108.00^{B}$ ± 3.929 | $2.00^{B}$ ± 0.152 | $6.400^{AC}$ ± 0.127 |
| Oxytetracyclin e treated (infected) | $15.00^{C}$ ± 1.020 | $1.100^{C}$ ± 0.032 | $145.00^{C}$ ± 4.472 | $2.200^{BC}$ ± 0.179 | $7.900^{B}$ ± 0.211 |
| Enrofloxacin treated (infected) | $13.00^{D}$ ± 1.265 | $0.400^{D}$ ± 0.051 | $140.00^{D}$ ± 3.453 | $2.400^{C}$ ± 0.127 | $6.900^{C}$ 0.245 |
| Levamisol treated (infected) | $18.00^{E}$ ± 1.265 | $0.760^{E}$ ± 0.031 | $102.00^{A}$ ± 2.417 | $2.100^{B}$ ± 0.117 | $8.500^{D}$ ± 0.196 |
| LSD (at $P \leq 0.05$) | 1.307 | 0.050 | 4.620 | 0.231 | 0.370 |

**N.B.** * The different letters in columns point to the significant change between means (at $p \leq 0.05$), * LSD = Least Significant Difference between means (at $p \leq 0.05$).

## DISCUSSION

The clinical signs of FMD infected buffaloes observed in Kaleubia Governorate were salivation, lamness, rise in temperature and later appearance of vesicles in and around the mouth and in the interdigital space, in addition to the drop of milk, feed consumption and body weight. A great variation in clinical signs was observed among infected animals depend on the immune status, the level of challenge and the efficacy of vaccine used *(28)*. The apparent clinical signs of the FMD-infected dairy buffaloes inspite of previous (9 month ago) vaccination, indicating failure of vaccine to combat recent infection where FMD virus of serotype-O was detected in infected buffaloes, and this may attributed to the virus may change its antigenic identity *(2)* or may due to presence of no cross immunity between serotypes and subserotypes where there are three major strains of FMD (A, O and C serotypes), but there are substrains (subserotypes) with different degrees of virulence within them. No cross immunity between FMD strains and substrains was previously recorded by *(29)*.Three additional strains are : $SAT_1$, $SAT_2$ and $SAT_3$ (in Africa) and a further strain ASIA-1 from far East. Unfortumately, the virus also capable of infinite mutation, so that new antigenically different subserotypes are constantly appearing *(30)*, so that this was the probable cause of failure of vaccination program in many countries, for that it is necessary to choose a vaccine contains strain (s) antigenically similar to the outbreak strains in the involved area (1).

The gamma globulins, WBCs count, lymphocyte percentages and the specific antibody titre against FMD (serotype, O) infection were significantly elevated by the levamisol treatment indicating that levamisol may be an immunostimulant drug to the FMD

infection and may be given to obtain maximum production of the total immune responses against the FMD- diseased animals. Oppositely, enrofloxacin treatment of FMD infected buffaloes showed significant decrease in antibody titre, gamma globulins WBCs counts and lymphocyte percentage than that of FMD-infected (+ve control) buffaloes, indicating the strong immunosuppressive effect of enrofloxacin treatment to the FMD infection. However, the oxytetracycline significantly decrease the specific antibodies against FMD infection but not induce decrease of gamma globulins and significantly increase the total lymphocyte percent compared to positive control group, indicating that the oxytetracycline is weakly immunosuppressive when compared with strong immunosuppressive effect of enrofloxacin. The immunostimulant effect of levomisol-Hcl treatment could be detected also in FMD-vaccinated buffaloes by ***Qureshi et al. (31)*** where the drug significantly rised the antibody titre until the 6th week post vaccination. The immunosuppressive effect of enrofloxacin treatment could be detected against brucella (infection or vaccination) in sheep by ***Helal and Abdel Fattah (32)*** and others. Also, the oxytetracyclin antibiotic previousely recommended as a preferable therapy during the coarse of Bovine Ephemeral Fever (BEF) viral infection, because it improves the hypogammaglobulinemia and lymphopenia induced by BEF diseased cattle ***(33).***

The RBCs and WBCs counts, the haemoglobin (Hb) concentration and the Mean corpuscular haemoglobin concentration are decreased in FMD infected buffaloes after treatment with either enrofloxcin antibacterial or oxytetracyclin antibiotic, but in contrast, these parameters were increased by levamisol treatment compared to FMD infected, non-treated (+ve control) buffalo group, indicating that the levamisol did not interfere with those blood parameters. In contrast, the oxytetracycline and enrofloxacin enhancing the decrease of such parameter perhaps attributed to the erythrocyte destruction as recorded by some chemicals, or to their effect on erythropoiesis as a result of defects in nucleic acid synthesis or deficiencies in vitamin-$B_{12}$, folic acid, iron, pyridoxine or copper which could induced also by some chemicals or antibiotic drugs ***(34),*** also the leucopenia may result from antibacterial administration or also due induction of deficiencies in vitamin-$B_{12}$, niocin, or folic acid ***(35).***

The present study showed that both the packed cell volume (PCV) and the mean corpuscular volume (MCV) are significantly decreased in all FMD infected buffaloes than that of the normal –ve control buffalo group. The PCV is also referred as the hematocrit, where it used for calculation of MCV and the mean corpuscular haemoglobin concentration (MCHC). The decrease of PCV will induced anaemia (hemolytic, hemorrhagic or defective erythroporesis) ***(36)*** as previousely discussed.

The α-and β-globulins increased significantly in FMD infected buffaloes than –ve control group, also the drug treatments (in most cases) induce more significant increase of the α and β-fractions compared to the +ve control group. ***Kaneko (37)*** reviewed that there are 13-types of α-fractions have been reported with some toxic chemicals ***(38).*** Some immunoglobulins of γ-fractions can rise to the β-fraction in response to antigenic stimuli ***(37),*** where infection also increase the immuno (γ) globulins which more stimulated significantly by levamisol treatment compared to other treatments and +ve control group, with parallel increase also of leucocytes and lymphocyte percentages and the specific antibody titres against FMD infection. The γ-globulins are synthesized in the plasma cells which maturated from B-lymphocytes in the spleen, bone marrow and lymph nodes ***(39)***.

The neutrophils decreased (mostly) by all FMD infected groups of buffaloes than that of the -ve control group. The neutropenia may be induced by salmonella infections, some toxic chemicals, some drugs, acute haemorrhages or leukemias ***(35).***

The monocytes were only increased by FMD infected non-treated (+ve control)

buffalo groups, but it decreased by enrofloxacin treated buffaloes than that of +ve control group. Monocytes (phagocytic cells found in the peripheral circulation) are related to similar cells found in the tissues (macrophages), and the monocytes may form macrophages when they migrate to the tissues, the monocytes not only responds to infection but also functions in destruction of damaged tissue and processes antigens in order to provoke a lymphocyte response ***(40),*** so that enrofloxacin treatment suppress the monocyte population and this strengthen its immundepressant activity as previousely discussed. The enhancement of monocyte population by FMD virus infection or vaccination could be recorded also by ***Khatri et al. (41).***

The present study revealed that, the activity of Alanine aminotransferase (ALT) enzyme and the concentrations of serum total bilirubin, total cholesterol and creatinine are significantly increased by FMD infected buffaloes than that of normal (-ve) control group, this increases are more significantly elevated by the all drug treatments compared to the positive control group, but this increase become non-significantly by levamisol and oxytetracycline (in case of creatinine) and by levamisol (in case of cholesterol). The total protein non-significantly changed by FMD infection, but the levamisol and oxytetracycline treatments could elevate the total protein compared to the positive control group. This indicate that the enrofloxacin treatment (in addition to its immunosuppressive effect) could induce also more hepatotoxicity and nephrotoxicity followed by the oxytetracycline then the levamisol which showed only heptotoxic effects (but not induced nephrotoxicity). The increased levels of ALT enzyme activity and the concentration of total bilirubin are usually occurred in case of acute hepatic necrosis, extrahepatic obstruction, congestive hepatomegalia, and infectious mononucleosis *(42).* The increased serum creatinine usually does not occur until the renal function is substantially impaired *(36),* also the hypercholesterolemia has been reported in liver or renal diseases, hypothroidism or diabetes mellitus *(43).*

Based on the current study, it could be concluded that there was a failure of vaccination program against FMD in Kaleubia Governorate, where the animals infected with FMDV (serotype-O) may attributed to the lack of cross immunity between different subserotypes, so that we could suggesting the use of attenuated living virus trivalent vaccines (against A, O and/or C-serotypes) instead of killed (inactivated) one, prepared from the local strains, because the short duration of the immunity produced by the latter vaccine, with eradication of diseased animals, and this action has proved effective in South Africa and Venezuela as recorded by *(29)* and *(44).* It could concluded also that the levamisol induced strong immunostimulant to FMD infection in buffaloes, oppositely, the enrofloxacin treatment induced strong immunosuppressive effects of FMD infected buffaloes, while oxytetracycline induced weak immunosuppressive effect with less toxicity compared to enrofloxacin. So that it could be suggested that the enrofloxacin should contraindicated in case of FMD infection or vaccination, but the oxytetracycline may be used from the 1st day of appearance of clinical signs, with a dose lower than the therapeutic one, as a prophylactic procedure against 2ry bacterial complications, also levamisol should be periodically injected with FMD infection or vaccination to elevate the total immune responses against the FMD disease, in order to shorten the period of infectivity and reduced its severity, and also for prolonging high or antibody titres of vaccinated animals.

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## الملخص العربى

## بعض الدراسات البيوكيميائية والهماتولوجية والفيروسية والمناعية على الجاموس المصرى المصاب بفيروس مرض الحمى القلاعية

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من خلال وباء مرض الحمى القلاعية الذى انتشر فى يناير عام ٢٠٠٦ بمحافظة القليوبية بمصر – تم الاختيار العشوائى لعدد خمسة وعشرون جاموسة حلابة من المزارع الخاصة حيث قسمت إلى خمسة مجموعات متساوية، المجموعة الأولى حيوانات غير محصنة وغير مصابة بأعراض المرض حيث استخدمت كضابط سلبى للدراسة، والمجموعات الثانية والثالثة والرابعة والخامسة فهى لحيوانات محصنة ضد الحمى القلاعية (العتره – ٠، منذ ٩ شهور سابقة للإصابة) ومصابة أيضا بأعراض مرض الحمى القلاعية، حيث المجموعة الثانية مصابة

ومحصنة وغير معالجة دوائيا واستعملت كضابط إيجابى للدراسة، والمجموعة الثالثة معالجة بالمضاد الحيوى، أوكسى تتراسيكلين والمجموعة الثالثة معالجة بالمضاد البكتيرى إنروفلوكساسين، والمجموعة الخامسة معالجة بمضاد الديدان "ليفاميزول" – كل المعالجات الدوائية تمت بالحقن العضلى مرة واحدة يوميا ولمدة خمسة أيام متتالية قبل أخذ العينات مباشرة. فى اليوم السادس من بدأ المعالجة الدوائية تم أخذ عينات من البلعوم والحويصلات المصلية والتقرحات واللعاب بالفم للاختبارات الفيرولوجية، وتم أخذ عينات دم للفحوص الهيماتولوجية، وتم أخذ عينات سيرم بغرض الفصل الكهربى لبروتين المصل وللفحوص السيرولوجية والبيوكيميائية. أوضحت نتائج الدراسة أنه تم عزل فيروس مرض الحمى القلاعية (العتره – O) بالرغم من تحصين القطيع بنفس العتره من تسعة شهور سبقت للعدوى بالمرض. وأوضحت نتائج المعالجات الدوائية أن المضاد البكتيرى "إنروفلوكساسين" مثبط مناعى قوى لمرض الحمى القلاعية، ولذلك فيجب منع معالجة الجاموس المصاب بالمرض بهذا العقار. وعلى العكس من ذلك فإن عقار "اليفاميزول" أوضح تنبيها مناعيا قويا ضد المرض وينصح دائما بحقنه فى الحيوانات المصابة بالحمى القلاعية لتقليل ضراوة وفترة المرض وعلى أى حال فقد أوضحت المعالجة بالأوكسى تتراسيكلين تثبيط ضعيف ضد المرض بالمقارنة بالتثبيط القوى ضده باستخدام عقار الإنروفلوكساسين، ولذلك يمكن اقتراح حقن الأوكسى تتراسيكيلين بجرعة أقل من الجرعة العلاجية منذ اليوم الأول لظهور أعراض المرض، كوقاية ضد المضاعفات البكتيرية، وبأقل آثار جانبية ممكنة. وينصح بتجريب استخدام لقاح الفيروس الحى المستضعف والمتعدد العترات والمحضر من العترات المحلية وذلك من أجل الحصول على أجسام مناعية بتترات عالية ولمدة أطول ضد مرض الحمى القلاعية مع الإعدام الصحى للحيوانات المصابة.