

## Effect of Foot and Mouth Disease in Egyptian Cows and Sheep on Characterization of Serum Lysosomal Enzymatic Activities

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### ABSTRACT

In this study, clinical findings, virus recognition and the activity of some lysosomal enzymes such as  $\beta$ -N- acetyl glucosaminidase ( $\beta$ - NAG) Acid phosphatase (ACP) and  $\beta$ -galactosidase ( $\beta$ - GAL) in serum of Egyptian dairy cows and sheep naturally infected by foot and mouth diseases were determined.

In addition, the lysosomal enzymatic activities in vaccinated animals were also performed. The results revealed that the enzymes activity of ACP was increased by 454% and 806% in vaccinated cows and sheep respectively, while the activity appeared to be less in infected animal either in cow by 182% or 580% in sheep.  $\beta$  -NAG activity exerted a relative lower of the percentage change by 3.3% and 99.0% either in cows or in sheep of vaccinated animals. While in diseased animals the enzyme activity of  $\beta$ - NAG was reduced by 5.6% for cows and increased by 767% for sheep. On the other hand, the enzyme activity of  $\beta$ - GAL appeared to be of moderate activity of enzyme release either in vaccinated or infected animals by 119%, 574% for vaccinated cows and sheep respectively and 54.7%, 1171% for diseased cows and sheep respectively. The obtained results revealed that the enzyme activity of the lysosome was altered according to vaccinated or diseased animals. ACP activity approved to be highly activity in the vaccinated cows than in the diseased ones. The activity of  $\beta$ - NAG in sheep exerted a highly percentage change more than in cows in both of vaccinated and diseased animals.  $\beta$ - GAL activity exerted a highly activity in the diseased animals more than vaccinated animals for sheep.

We have concluded that the lysosomal enzymatic activity was highly increased in sheep more than in cows either vaccinated or diseased animals. This study might be open the route for using the lysosomes as adjuvant with FMD vaccine or for increasing the protection against FMD.

**Key words:** Lysosmoes; Foot and mouth diseases; Vaccinated and infected cows and sheep.

### INTRODUCTION

The use of lysosomal enzymes received little attention in veterinary field in Egypt, although (1) performed an experiment on Holstein dairy cattle fed on dairy urea in Faculty of Veterinary Medicine, Zagazig University paved the way under the acid hydrolases lysosomal enzyme. (2) Undertook the estimation of acid hydrolyses lysosomal enzymes in the serum for diagnosis and prognosis of lumpy skin disease in Egyptian

cows in Ismalia province. (1) Continued the amelioration of the way in Egypt under the use of the same lysosomal enzymes in serum in an outbreak of Equine Influenza in Menofia province.

Lysosomes are organelles destined to degradation and recycling in body cells leading to cellular hemostasis (3&4). Genetic program controls the lysosomal biogenesis providing the hallmark to increase the value of cellular

clearing in the lysosomal storage disorders and neurodegenerative diseases (5).

As inflammation could affect the lysosomal enzymes (6), Foot and Mouth disease causes inflammation through the virus and its secondary bacterial infection during vesicle formations and their eruptions (7).

FMD is highly acute contagious and economically devastating viral disease of cloven footed animals because of rapid spread, weight loss, mastitis, loss of milk production and frequent abortion (8). Hand Foot and mouth disease (HFMD) is a disease of infant and children. Human and animal are tolerant to each other (9). FMD is an endemic in Asia, Africa and South America (10). It infects cattle, swine, sheep and goats, other domestic and wild cloven footed animals (11).

The cause of FMD is single- stranded positive – sense RNA virus which belongs to Aphthovirus genus of the Picornaviridae family (12). There are seven distinct serotypes A, O, C, Asia 1, South African Territories SaT1, SAT2, SAT3 and they are immunological distinct (13&14). (15) Stated that FMD is the most economically important disease of livestock worldwide. Foot-and mouth disease virus serotypes O, A and Asia 1 are endemic or cause periodic FMD outbreaks in the Middle East and serotypes O and A cause FMD outbreaks in North Africa (16-18). This region is also threatened by sporadic incursions of different FMD serotypes that are normally restricted to Sub-Saharan Africa (19).

During 2012, there has been a dramatic upsurge in FMD SAT 2 outbreaks in Egypt. Initial cases were recognized in the Delta Governorates (Gharbia and Sharkia) and Alexandria, and further outbreaks of disease were also suspected in Upper Egypt including Sohag, Qena and Aswan Governorates. Cattle, water buffalo and small ruminants were affected with severe clinical signs of FMD particularly in young animals where a mortality rate of up to 50% was observed due to multifocal myocarditis (20).

According to (7) the clinical signs of cattle are fever 39.4- 40.6°C, depression,

anorexia and disinclination to move, drop of milk yield and excessive drooling of saliva which hangs in long ropy strings causing lip smacking. Serous nasal discharge, shaking of head, kicking of the feet and lameness. The author also recorded vesicles formation (blisters) on the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary bands and teats. Abortion of the pregnant cows and young calves may die without developing any vesicles mainly from myocardial necrosis . FMD course needs 2-3 weeks. Secondary infection may delay recovery. The lactating cows may not recover due to the damage of secretory tissue. (21) Reported mastitis as the complication of FMD in dairy cattle. Clinical signs in sheep are mild including dullness and formation of small vesicles or erosions on the dental pad, lips, gums and tongue. Mild lameness with vesicle and erosions on the coronary band or in the interdigital space. Infected animals may abort. Nursing lambs may die without any clinical signs.

Autophagy is considered very useful for protection against cell aging and against variable diseases of animals as well as human diseases like cancer, cardiac diseases, diabetes, neurodegenerative and infectious diseases. Also it is assured that the autophagy is lysosomal process involved in the maintenance of cellular homeostasis, which is responsible for the turnover of long- lived proteins and organelles which were either damaged or functionally redundant (22).

It is worth noting that some of lysosomal enzymes are constituents of membrane as B-N-acetyl glucosaminidase and B- glucosidase enzymes. The portion of the soluble enzyme is bound to the membrane while (23) had the proof that acid phosphatase enzyme occurs outside the lysosome .

The release of lysosomal enzymes occurs before death of the cell therefore compounds which antagonize or reduce this release prevent the cell death and they called cell stabilizers (3&24).

This study is a trial to point a gun at the effect of FMD in Egyptian dairy cows and sheep on the lysosomal enzymatic activities of Acid phosphatase ACP,  $\beta$ - N- Acetyl glucosaminidase " $\beta$ - NAG" and  $\beta$  galactosidases " $\beta$ - GAL" in the serum of diseased and vaccinated animals.

## MATERIAL AND METHODS

### Animals and samples

One thousand six hundred and thirty two "1632" dairy cows and "84" sheep were selected from different farms and localities at Sharkia and Ismailia governorates. Negative dairy cows weighed 450- 530 kg each and native sheep weighed 68-100kg.

Dairy cows are fed on base diet containing wheat bran, cotton seed cake, yellow corn, molasses, sodium chloride (common salt), calcium carbonate (lime salt) and curde protein.

Sheep are fed on green ration of *Trigolium Alexandrium*, dry ration of Hay and concentrate.

Concentrate includes 60% corn, 20% cotton seed cake, 77% wheat bran, 2% calcium chloride and 1% Na Cl.

Animals were divided into:

- 1- Control group "10 apparently healthy cows and 10 sheep" .

- 2- FMD diseased cows and sheep "naturally infected" 1602 cows were examined, only 22 cows were suffered from FMD and 64 sheep were examined and only 7 sheep were suffered from FMD.

- 3- Vaccinated cows "20" and sheep "10" with FMD international imported vaccine (Raksha- Ovac) which contains in-activated FMD virus strain O1, A22 and A96 adjuvanted with mineral oil. Complete inactivation of the virus is ensured by the use of Azirdine compound. The vaccine is produced by Indian Immunological Limited Company ( Jubile Hills, Hyderabad, India). Route of vaccination is subcutaneous. Dose: first dose 2 ml. booster dose 2 ml with 4 weeks interval.

Identification of the previous or current infections

All collected sera from cattle and sheep were tested by the non structural proteins (NSPS) antibody test using Chekit- FMD-3ABC bo-ov ELISA kit (Bommeli Diagnostics Liebefeld- Berin, Switzerland) to identify the previous or current infections with any serotypes of the virus, whether or not the animal has also been vaccinated (table, 1). Therefore the test can be used to confirm suspected cases with FMD and to detect viral activity or to ensure freedom from infection on a population basis. The test procedure was carried out as described by the manufacture. The test is a blocking ELISA which measures the competition between test ser and a NSP specific monoclonal antibody for the binding to the 3 ABC NSP of FMDV according to (25)

**Table 1. Identification of the previous or current infection by using Chekit- FMD-3ABC bo-ov ELISA kit in cattle and sheep**

Farms and localities	No. of tested serum samples	No. of negative 3ABC	No. of Positive 3ABC	No. of positive 3ABC showed mild clinical findings (%)
Cattle Farm I (Sharkia governorate)	812	800	12 (1.5%)	4 (33.33%)
Farm II ( Ismailia governorate)	790	780	10 (1.3%)	3 (30%)
Total	1602	1580	22 (1.4%)	7 (31.8%)
Sheep Six Localities*	64	57	7 (10.94%)	2 (28.57)

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## Lysosomal enzymatic activities assay "LYE"

Blood samples were collected for serum separation to determine the enzyme activity of the lysosomal enzymes ",  $\beta$ - NAG, ACP and  $\beta$ - GAL" from apparent healthy, naturally affected and from the vaccinated animals" 3 days after the vaccination" which measured spectrophotometrically according to the method of (26) with some modification by (27).

The substrate of lysosomal enzymes are:

- 1) P. nitrophenyl phosphate " sodium salt" was used for orthophosphoric monoester phosphohydrolases (Acid phosphate) "E.C.3.1.3.2"
- 2) P.niteophenyl-2-acetamido- 2- deoxy-  $\beta$ - glucopyranoside was used for N- acetyl  $\beta$ - D- glucosaminidase "E.C.3.2.1.30."
- 3) P.nitrophenyl-  $\beta$ - D- galactopyranoside was used for  $\beta$ - galactosidase " E.C.3.2.23"

## Statistical analysis

Levels of significance of difference between means of treated sample and control were stastically evaluated by the use of the non paired student (t) test (28).

## RESULTS AND DISCUSSION

From clinical examination Cows showed dullness, disinclination to move, anorexia, sluggish manner and drop of milk yield. Fever varied in different farms from its absence, unnoticed to high fever. Vesicles appeared on mucous membranes, the borders and dorsum of the tongue, buccal sides of cheeks, inner sides of lips, gums and margins of dental pad as well as angles and margins of mandible. Evidence of vesicles on feet was present. The lesions (vesicles) had the tendency to stabilize or go into remission (rarely) or go into eruption (commonly). At the last visit abortion, mastitis and pneumonia are recorded. Our discernible clinical findings of cows are in coincidence with (21) and plenty of authors in all the world because FMD occurs in almost every country where cows are kept.

About the clinical findings in sheep, it seems to be milder than in cows. According to our clinical signs and the report of (29) viraemia is recorded. There were not vesicles but fever, dullness, sluggish manner and lameness in ewes were observed. In pregnant ewes, abortion occurred with dead lambs. The transplacental infection which was amenable to treatment lead to dead fetal lamb (30).

Outcomes of deaths in other lambs were due to circulatory, respiratory collapse, tachycardia and fever. These results were in coincidence with (31).





Picture "1"



picture "2"

Picture 1. Shows rosy salivation and erupted vesicles with presence of necrotic epithelial tissue while picture "2" shows erosion and erupted vesicles at interdigital space.

Table 2. Effect of foot and mouth disease on lysosomal enzyme activities in Egyptian dairy diseased cows and sheep:

Lysosomal enzyme	Control of dairy cows	Control of sheep	Lysosomal enzymatic activities by nmol/ml/hr (Mean±S.E)	
			Diseased	
			Cow	Sheep
β - NAG % Change	4438±1.90	616.7±0.011	4191.5±0.31* ↓5.6%	5350.9±0.150* ↑767%
ACP% Change	228±15.6	146.9±0.006	643.6±0.046* ↑182.3%	998.9±0.091* ↑580.2%
β - GAL% Change	384±17.0	98.89±0.003	594.0±0.028* ↑54.7%	1257.6±0.041* ↑1171.7%

\*P<0.05: as compared with control group.

Table 3. Lysosomal enzyme activities in vaccinated dairy cows and sheep

Lysosomal enzyme	Control of dairy cows	Control of sheep	Lysosomal enzymatic activities by nmol/ml/hr (Mean±S.E)	
			Vaccinated	
			Cow	Sheep
β - NAG % Change	4438±1.90	616.7±0.011	4583.5±0.31* ↑3.3%	1227.3±0.036* ↑99.0%
ACP% Change	228±15.6	146.9±0.006	1263.6±0.051* ↑454.2%	1330.4±0.068* ↑806.0%
β - GAL% Change	384±17.0	98.89±0.003	842.4±0.0043* ↑119.4%	666.7±0.007* ↑574.2%

\*P<0.05: as compared with control group.

The results gathered from table (2) showed that the activity of  $\beta$ -NAG showed decreased activity in dairy cows with 5.6% when it is compared with the control 4438nmol/hr which descend to 4191.5 nmol/hr, with FMD virus, this may be due to presences of antioxidant.

Lysosomal enzymatic activities reduction was supported with the findings obtained by (32) who reported that a decrease of lysosomes in serum is as a response to general virus infection in human beings. (33) Recorded increased free lysosomes in the non bacterial meningitis in a pediatric research. This increment is conceptualized here with our sheep serum lysosomal enzymatic activities results which increased with ragumented quantities from 616.7 nmol/hr in control sheep to 5350.9nmol/hr in natural FMD infected sheep with 767.7%. The lysosomal increase was also reported by (34) in diagnosis of rheumatic arthritis in human. This high content of lysosomes is corroborated with (35-41) who suggested that the viral insult may be responsible for the acceleration of T- cell proliferation.

Acid phosphatase in the present study was higher in the vaccinated animals than in the naturally infected (diseased) by 1263.6 nmol/hr, 1330.4 nmol/hr and 643.6 nmol/hr, 998.9 nmol/hr for vaccinated and diseases cows and sheep respectively.

The lifting up to higher level may be due to increase in the synthesis by rough endoplasmic reticulum (41).

$\beta$  - GAL activity in naturally diseased infected animals have lower values in cows 594 nmol/hr than in sheep which showed the highest activity 1257 nmol/hr which required more researches and veterinary investigations.

Efficacy of vaccination of dairy cows and sheep will give the chance to the vaccine used to have a tight relationship between lysosomal enzymes and antibody formation in addition to its success in veterinary field in Egypt. Lysosomal enzymes were increased expressing the formation of antibodies against FMD virus.

The absence of available literature to compare the results of vaccinated cows and sheep which verified the percentage increase in this study 3.3% and 99% respectively than that of control animals, (40) reported higher enzymatic activities of acid hydrolases ( $\beta$ -glucuronidase, acid phosphatase and Catgepsin) after infection of laboratory animals with attenuated strain of tubercle bacilli, BCG. (41) Attributed the lifting up of lysosomal activity to the regulatory genetic coding which goes with increment of enzymatic activities induced or mediated by prostaglandin synthesis.

## CONCLUSION

Foot and Mouth disease is an endemic disease in our country which threaten our animals, by introducing new strain, so much work is needed to control and prevent this disease.

The lysosomal activity must be coordinated to respond to cellular needs. This response remains a subject of debate and need further works. We hope that our study will generate a lysosomal deal of interest such as to spearhead new researches into different animals.

Further studies are needed to clarify the mechanisms which are responsible for the effect of FMD on lysosomal activities. In addition the possibility of usage of lysosomes as adjuvant with FMD vaccine.

Lysosomal activities of the three selected enzymes "  $\beta$  -NAG, ACP and  $\beta$  -GAL and " were highly increased in diseased and vaccinated animals except  $\beta$  -NAG was decreased in diseased than control dairy cows and this increasement was high in vaccinated than in diseased sheep and cows.



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### الملخص العربي

#### تأثير مرض الحمى القلاعية علي انزيمات الليزوزم في الأبقار والأغنام المصرية

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في هذا البحث تم دراسة تأثير مرض الحمى القلاعية علي نشاط بعض انزيمات الليزوزوم مثل  $\beta$ -N-acetyl galactosidase "  $\beta$ -GAL" و Acid phosphatase "ACP" و  $\beta$ -NAG glucosaminidase في سيرم الأبقار والأغنام المصابة بالحمى القلاعية والمحصنة ضد نفس المرض ولقد لوحظ من هذه الدراسة أن أكثر الانزيمات تغيراً هو ACP حيث زاد معدله الي ٤٥٤% و ٨٠٦% في الأبقار والأغنام المحصنة علي التوالي بينما كانت الزيادة أقل في الحيوانات المصابة حيث وصلت الي ١٨٢% و ٥٨٠% في الأبقار والأغنام علي التوالي مقارنة بالحيوانات السليمة.

بينما انزيم  $\beta$ -NAG أوضح تغيراً أقل حيث زاد معدله الي ٣,٣% و ٩٩% في الأبقار والأغنام المحصنة علي التوالي بينما قل نفس الانزيم في الأبقار المريضة وزاد في الأغنام المريضة بمعدل ٧٦٧%.

وقد أوضحت النتائج تغيراً متوسطاً في انزيم  $\beta$ -GAL حيث زاد بمعدل ١١٩% و ٥٧٤% في الأبقار والأغنام المحصنة و ٥٤٧% و ١١٧١% في الأبقار والأغنام المريضة علي التوالي.

وقد أوضحت الدراسة أن مستوي الانزيمات يتغير في الحيوانات المريضة عن الحيوانات المحصنة ولوحظ زيادة معدلها في الحيوانات المحصنة عن الحيوانات المريضة وفي الأغنام أكثر منها عن الأبقار.