

Heat-Intolerance Syndrome subsequent Foot-and-Mouth Disease in Cattle; ThiQar –Iraq

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Summary

The investigation on Heat-Intolerance Syndrome following foot and mouth disease (FMD) infection in cattle in ThiQar-Iraq, used 3ABC FMD ELISA kit, and Radio- immunoassay (RIA) to detect the cortisol level. From 105 there were 65(62%) infected cattle with FMD, which was high at 5- less 8 years old, while the cortisol level showed three levels; normal (13-21 nmol/L), high and low levels were; 6(5.6%), 44(42%) and 55(52%) subsequently. More over the combined result of ELISA and RIA had divided cattle into six groups. First group of 40(38%) cattle infected with FMD and had low level of cortisol, this group containing 21(20%) with clinical signs of heat intolerance. Second group contain 22(21%) FMD infected cattle with high level of cortisol. Third group of 3(2.8%) FMD Infected cattle but normal cortisol level. Fourth group included 3(2.8%) not infected by FMD with normal cortisol level. Fifth group contain 22(21%) not infected with FMD but had high level of cortisol may related to stress. Sixth group consisted 15 (14%) cattle not infected with FMD and had low level of cortisol due to un known cause.

Clinical signs of heat intolerance that showed in 21 head of cattle in the first group were: panting, overgrowth of hair coat, emaciation and seeking for shade. The diseased cow known locally as "Mahrorah" meaning heat-intolerance.

Keywords: Heat-Intolerance , FMD, Cattle, ABC- ELISA, RIA, cortisol, ThiQar, Iraq

متزامنة عدم تحمل الحرارة المرافقة للإصابة بالحمى القلاعية في الأبقار: ذي قار العراق

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الخلاصة

عدت هذه الدراسة للتحري عن متزامنة عدم تحمل الحرارة بعد الإصابة بمرض الحمى القلاعية في الأبقار في محافظة ذي قار ، وذلك باستخدام عدة الأليزا (3ABC FMD ELISA kit) وفحص هرمون الكورتيزول بالتحليل الاستشعاعي الممتنع لمصل الدم من خلال فحص (105) رأسا من الأبقار وجد (65)% (62) مصابا بالحمى القلاعية وكانت الإصابة في أوجها بعمر من 5- أقل من 8 سنوات بينما كان مستوى هرمون الكورتيزول الطبيعي 21-13 مل مول وظهر في الأبقار بمستويات من الأعلى إلى الأقل على التوالي: (6)(55%) و (44%) و (42%) و (52%) علاوة على ذلك إن ربط علاقة نتائج الكشف بالـ ليزا مع مستوى الكورتيزول وزعت الأبقار إلى ستة مجاميع:المجموعة الأولى من (40) رأسا (38%) أبقار مصابة بالحمى القلاعية وتحمل مستوى واطئ من هرمون الكورتيزول وقد اشتملت هذه المجموعة على (22) رأسا (21%) يعاني من علامات سريرية لعدم تحمل الحرارة . والمجموعة الثانية من (22) رأسا (21%) أبقار مصابة بالحمى القلاعية وتحمل مستوى عال من الكورتيزول . والمجموعة الثالثة من (3) رأسا (2,8%) أبقار مصابة بالحمى القلاعية ولكنها تحمل مستوى طبيعي للكورتيزول ، والمجموعة الرابعة شملت (3) رأسا (2,8%) أبقار غير مصابة بالحمى القلاعية وكذلك ذات كورتيزول بمستوى طبيعي ، والمجموعة الخامسة تألفت من (22) رأسا (21%) أبقار غير مصابة بالحمى القلاعية ولكنها تحمل مستويات عالية من الكورتيزول ربما بسبب الإجهاد، والمجموعة السادسة كانت من (15) رأسا (14%) أبقار غير مصابة بالحمى القلاعية مع ذلك تحمل مستوى واطئ للكورتيزول لأسباب غير معرفة. وتتميز العلامات السريرية لعدم تحمل الحرارة في 21 رأسا من الأبقار ضمن المجموعة الأولى باللهاث وزيادة نمو شعر الجسم والهزال والبحث عن الظل وعرفت البقرة المصابة بالمرض بين القرىتين في تلك المناطق بالمحرورة وتعني عدم تحمل الحرارة .

Introduction

Foot-and-mouth disease (FMD) is highly contagious viral disease of cloven-hoofed domestic and wild animals. It is widely distributed and occurs most commonly in Asia and Africa (1). FMD is caused by a virus of the Aphthovirus genus within the family Picornaviridae (2). There are seven FMDV serotypes O,A,C, Asia-1, South African Territories types (1,2 and 3) and immunity to one does not cross-protect against the others(3).

The FMD virus serotypes that detected in Iraq were; serotype A since 1952, O in 1957 , SAT 1 in 1962 and Asia 1 in 1975, but most recent outbreaks were due to O and A strains during years 2000 – 2009 (4 ; 5 , 6). The FMD WRL (Pirbright, UK) report suggests the last outbreak was caused by emerging strain A-Iran-05 (7).

As a sequel of foot-and-mouth disease, a proportion of infected cattle develop dyspnoea, long and rough coats, poor body condition and chronic, irreversible impairment of production potential. These cattle are colloquially known as "panthers "in India (8). In the late 1990s, a syndrome of heat intolerance and overgrowth of hair in cattle was reported by workers in Morogoro region, Tanzania (9). Cattle with Heat Intolerance manifested by loss body condition, and reduced milk production (10).

So This study aimed to determine the Heat Intolerance Syndrome as a sequel of foot-and - mouth disease in cattle in ThiQar province.

Materials and methods

Equipments;

- Microplate washer / Gesellschaft für Biochemica and Diagnostica / Germany
- ELISA Reader / Dia Med Euro Gen / Belgium..
- RIA gamma counter/Block Scientific/ Englewood,NJ 07631

Cattle; 105cattle were chosen in a period distended from 7 to 26 October 2009, from ten villages at four- directions in ThiQar (north, east, south and west).

Blood Samples; were collected from a jugular vein of cattle into 10 ml sterile vacuum tubes without anticoagulant, serum separated by centrifuging (3000rpm for 5 minutes). (TABLETOP / Germany), then stored at -20C° until assayed.

Confirm the FMD infection by using **3ABC-Ab FMD ELISA kit** (Svanova /Sweden), to differentiate the naturally infected animals from those vaccinated with the FMD virus , and the procedure was according to company instruction, and corrected OD values(ODcorr) as the following equation;

$$OD_{Nsp3ABC} - OD_{control} = OD_{corr}$$

While the percent positivity Value (PP) as in;

$$PP = \frac{Test\ Sample\ or\ Neg\ (ODcorr)}{Positive\ Control\ (ODcorr)} \times 100$$

Then the Interpretation of test sample result as below;

Serum	PP	Interpretation
	< 48	Negative
	≥ 48	Positive

Estimation of cortisol level by using **Cortisol RIA kit**.(Beckman Coulter Company), the assay procedure was done according to company instructions.

Results

Among 105cattle of the studied population from ThiQar province there were 65(62%) positive for ELISA , the infection was high in age group 5-<8 years old 20(19.1%) (table1).

Table(1): The result of ELISA according to the age group of cattle

Cattle		ELISA Positive		ELISA Negative	
Age(years)	number	number	percentage	number	percentage
1-<2	21	14	(13.3%)	7	(6.6%)
2-<5	25	16	(15.2%)	9	(8.6%)
5-<8	32	20	(19.1%)	12	(11.4%)
8and over	27	15	(14.3%)	12	(11.4%)
The total	105	65	(62%)	40	(38%)
P-value= 0.869 (P > 0.05)		X² =0.715		degree of freedom(Df) = 3	

From the total of 105 serum samples of cattle measured by RIA, the cortisol increased in 44(42%) samples while it was low in 55(52%), but the normal were found in 6(5.7%) as shown in the table (2) .

Table(2): RIA Result of cortisol

Cattle		High level of Cortisol (> 0.75		Low level of Cortisol (<		Normal Level Of Cortisol (0.47-0.75) μ g /	
Age(years)	number	number	percentage	number	percentage	Number	Percentage
1-<2	21	11	(10.5%)	7	(6.5%)	3	2.9%
2-<5	25	15	(14.3%)	9	(8.5%)	1	0.9%
5-<8	32	10	(9.5%)	21	(20%)	1	0.9%
8and over	27	8	(7.7%)	18	(17%)	1	0.9%
The total	105	44	(42%)	55	(52%)	6	5.6%
P-value= 0.028 (P< 0.05)		X² =9.027		Df = 3			

The combination between ELISA & RIA Results to six groups illustrated in tables(3, 4,5,6,7 and 8):

Table (3): first group ELISA positive and cortisol at low level, and 21 cattle with a clinical findings of heat intolerance.

Cattle		ELISA Positive and Cortisol at low level(< 0.47 μ g / dL)		Cows with Clinical findings		Cows without Clinical findings	
Age (years)	Number	Numb er	Percentage	No.	Percentage	No.	Percentage
1-<2	21	6	(5.7%)	1	(0.9%)	5	4.7%
2-<5	25	7	(6.6%)	4	(3.8%)	3	2.9%
5-<8	32	16	(15.2%)	11	(10.5%)	5	4.7%
8and over	27	11	(10.5%)	5	(4.8%)	6	5.7%
The total	105	40	(38%)	21	(20%)	19	18%
Pvalue=0.609> 0.05		X² =1.825		Df = 3		Pvalue=0.646 > 0.05	
						X² =1.659	
						Df = 3	

Out of 40 cattle of the first group, there were 21 cattle had signs of heat intolerance that given in table (3), included stopped grazing and eating, loss body weight. It is also founded that for most time preferred to stand under the shade and dose not tolerated sun light. Close examination showed panting with respiratory rate(68-76) per minute and body temperature(39.5-39.7)C°, also there was overgrowth of hair coat particularly at the areas like withers, chest, along the back and on the flanks and easily determined the ribs which revealed emaciation and loss of body weight, such animals the farmers called them " Mahrorah".

Table (4): The second group with ELISA posetive and cortisol at high level

Cattle		ELISA Positive and Cortisol at high level (> 0.75 μ g / dL)	
Age (years)	Number	Number	Percentage
1-<2	21	6	(5.7%)
2-<5	25	8	(7.6%)
5-<8	32	4	(3.8%)
8 and over	27	4	(3.85%)
The total	105	22	(21%)
P-value=0.399 > 0.05		X² =2.948	Df = 3

Table(5): The third group ELISA Positive and Cortisol at Normal level

Cattle		ELISA Positive and Cortisol normal (0.47-0.75) μ g / dL	
Age (years)	Number	number	percentage
1-<2	21	2	(1.9%)
2-<5	25	1	(0.9%)
5-<8	32	zero	(0%)
8 and over	27	zero	(0%)
The total	105	3	2.8%
P-value=0.188 > 0.05		X² =4.778	Df = 3

Table(6): The fourth group ELISA Negative and Cortisol Normal

Cattle		ELISA Negative and Cortisol Normal (0.47-0.75) μ g / dL	
Age (years)	numb er	Number	Percentage
1-<2	21	1	(0.9%)
2-<5	25	zero	(0.0%)
5-<8	32	1	(0.9%)
8 and over	27	1	(0.9%)
Total	105	3	(2.8%)
P-value=0.781 > 0.05		X² =1.042	Df = 3

Table (7): The fifth Group, ELISA Negative and Cortisol at high level

Cattle		ELISA Negative and Cortisol at high level (> 0.75 μ g / dL)	
Age (years)	Number	Number	percentage
1-<2	21	5	(4.7%)
2-<5	25	7	(6.7%)
5-<8	32	6	(5.7%)
8 and over	27	4	(3.8%)
The total	105	22	(21%)
P-value=0.797 > 0.05		X² =1.014	Df = 3

Table (8): The sixth group ELISA Negative and cortisol at low level

Cattle		ELISA Negative and Cortisol at low level (< 0.47 μ g / dL)	
Age(Years)	Number		
1-<2	21	1	(0.9%)
2-<5	25	2	(1.9%)
5-<8	32	5	(4.7%)
8 and over	27	7	(6.6%)
The total	105	15	(14%)
P-value=0.263 > 0.05		X² =3.981	Df = 3

In association between ELISA and cortisol with exception of normal cortisol results that mean first(table-3), second(table-4), fifth(table-7) and sixth(table-8) groups were included as in table(9), whereas significant negative correlation (P < 0.05) between FMD antibody titter and serum cortisol level.

Table(9) FMD ELISA results and cortisol (low and high) level associations

ELISA Cortisol	ELISA positive	ELISA negative	Total
Low level (group)	40* (First)	15 (sixth)	55
High level (group)	22 (Second)	22 (fifth)	44
Total	62	37	99
P-value=0.02 < 0.05 *		X²=5.395	Df = 1

Discussion

The investigation about heat intolerance syndrome in ThiQar province by using 105 head of Cattle was disclosed a clinical findings in 21(20%) cattle which included panting , high body temperature, overgrowth hair coat and loss of body weight were also seen by (11) following FMD infection as a sequel called heat intolerance.

The high temperature of 39.5-39.7°C was related to imbalance between heat gain and heat loss, the haemostatic responses such increased respiratory rate and more obvious panting to compensate elevated temperature were overwhelmed (12). The heat regulation function in hypothalamus was damaged by FMD virus (13) , more over the loss of body weight for reason that affected cow was stop grazing, eating and walking to food, then became starved and emaciated (14).

The ELISA result from 105 there were 65(62%) FMD infected cattle, that in respective to serotypes, the ratio was relatively nearer to previous study by (15) whom founded infection ratio 61,42% in the middle and south of Iraq as endemic regions.

The used RIA for 105 cattle appeared three levels of cortisol ; high level(more than 21nmol/L) in the 44(42%) cattle which may related to stress a procedure of jugular blood collection for serum, that (16) had demonstrated that animals were significantly stressed by routine husbandry procedures . the low level (less than 13nmol/L) in 55(52%)cattle were also exposed to same procedure by blood collection method whereas not responded , given an indication to damage on endocrine system , because corticotrophic releasing hormons was released from the hypothalamus into the portal veins of the pituitary gland and adrinocorticotropic hormone secreted from the frontal lobe into blood stream which stimulated the adrenal cortex by releasing cortisol (17).

Normal level of cortisol (13-21nmol/L) was founded in 6(5.6%) cattle which were not stimulated by stress procedure of blood collection (18), and some cattle had adaptive coping

by endogenous pain inhibitory system, as well as phenomenon termed stress- induced analgesia (19).

The combination between ELISA and RIA results reviled four groups of cattle, first group with ELISA positive and cortisol at low level, included 40(38%) head of previously infected cattle by FMD virus, the reduction of cortisol level was also observed in primary or secondary hypoadrenocorticism, which manifested by low cortisol production and blood levels resulted from low adrenal cortisol released in response to adrinocorticotropic hormones(ACTH) stimulation because of the factors such as low adrenal mass and atrophy or destruction of the adrenal cortices. On other hand , the secondary hypoadrenocorticism may result from low pituitary ACTH production (20), so their is possible of secondary hypoadrenocortism is the cause of low cortisol level as several report have demonstrated FMD virus replication in pituitary gland of affected animals and significant reduction in blood cortisol level in cattle with heat intolerance syndrome which also agreement with (11).

The second group with ELISA positive and cortisol at high level, included 22(21%) head of previously infected cattle by FMD virus and recovered as a carrier which were stressed by procedure of blood collection, in agreement with (21 , 22).

The third group ELISA Positive and Cortisol at normal level included 3(2.8%) head of previously cattle were infected with FMD virus then recovered from the disease to become a carrier , such reason was explained by (23) who was differentiated the carrier state of FMD in cattle by asymptomatic persistent infection, followed the acute face of infection, but the recent group was not stressed by the procedures of blood collection.

The fifth Group, ELISA Negative and Cortisol at high level contain 22(21%) cattle not infected previously by FMD virus, those cattle may stressed by procedures of blood collection , similar stress was defined by (24) as a physiological response to events perceived as a potentially or actually threatening the integrity of the animal body , whereas serum cortisol was used as stress indicator when demonstrated by elevating blood cortisol level in mammal species.

The sixth group ELISA Negative and cortisol at low level included 15(14%) cattle were not infected by FMD virus for at least one year ago until time of blood collection due to their immunity which resist FMD infection but they may suffer from another disease state that reflected by decreased level of cortisol in their serum although the cattle were stressed by procedure of blood collection(11, 20).

According to Table(9) the correlation between FMD infected or non and abnormality of cortisol levels appeared that infected cattle by FMD virus like first group were significantly affected by decreased cortisol levels may due to damaged their pituitary gland (20), on other hand result was reversed by high cortisol levels for non infected cattle in the present study

In conclusion the cattle in ThiQar province had incidence of FMD virus infection of 62% also there was 38% of cattle in the endemic region were suffer from heat intolerance syndrome with low serum cortisol level as well as most of them had Clinical signs: panting, overgrowth of hair coat, emaciation and seeking for shad, which well known locally as "Mahrorah" cow, meaning heat-intolerance.

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