DEMONSTRATION OF AEROBIC SPORE FORMERS IN SOME MEAT PRODUCTS

Hemmat, M.I.[1], Amani, M.S.[1], Dalia, A.S.[2], Ghada, A.A.[2]

[1]Food Control Dept., Fac. Vet. Med., Benha Univ. [2] Animal Health Research Institute, Shebin El Koom Branch.

ABSTRACT

Aerobic spore formers especially *Bacillus* species are mainly be associated with food poisoning and among the main spoilage organisms in food due to their versatile metabolism and heat-resistant spores. A total of 100 random samples of meat products represented by (frozen rice kofta, kobiba-shami, oriental sausage and beef luncheon (25 for each) were collected from different shops, supermarkets and hypermarkets at Menoufia and Kalyobia governorates. These samples were examined bacteriolgically for presence of aerobic spore formers. The mean values of total aerobic spore forming counts in frozen rice kofta, kobeba-shami, oriental sausage and beef luncheon were $1.37\times10^3 \pm 0.25\times10^3$cfu/g, $1.16\times10^3\pm0.25\times10$, $1.03 \times10^3\pm0.17\times10^3$ and $8.58\times104\pm1.62\times104$ at 32°C and $6.14 \times10^2 \pm0.88\times10^2$, $6.52\times10^2\pm1.07\times10^3$, $4.79\times10^2 \pm 0.54\times10^2$ and $2.96\times10^2 \pm 0.31\times10^2$ cfu/g at 55°C, respectively. The highest incidence of aerobic spore formers was recorded in frozen rice kofta (88%) at 32°C and 84% at 55°C, while beef luncheon showed the lowest incidence (72%) at 32°C and 56% at 55°C. *Bacillus cereus* was the most predominant aerobic spore former contaminated such examined samples.

KEY WORDS: Aerobic spore formers, *Bacillus cereus,* Meat products.

(BVMJ-26(2): 219-226, 2014)

1- INTRODUCTION

Meat and meat products are considered as excellent sources of high quality proteins containing most essential amino acids that build and repair body tissues for maintenance of life. Meat also contains minerals and vitamins (Abd-Allah, 2005).

Meat and meat products are ideal for many organisms to grow because they are high in moisture, rich in nitrogenous compounds (amino acids, peptides, proteins) and plentifuly supplied with minerals and accessory growth factors. Furthermore, they have some fermentable carbohydrates, usually glycogen and keep favorable pH for growth of most microorganisms (Galvaz *et al.,* 2010). These constituents promote the growth and multiplication of various organisms including meat borne pathogens such as aerobic spore formers that may constitute public health hazards.

Technological development in meat processing, preservation and handling have given consumers much greater choice over the food they can buy. So, meat hygiene comprises very important in every aspect of processing from the health of the living animal to the distribution of the final product, it prevent harmful ingredients to be used in manufacturing of meat products and the sale of contaminated or unwholesome meat (Soliman, 2013). Moreover, lack of the sanitary measures during processing, handling and storage may act as the main source of food contamination with aerobic spore formers (Torky, 2004).

Aerobic spore formers have epidemiological interest, as some of their members are pathogenic and may result in serious infections and food poisoning. Moreover, the total number of these organisms can be taken as an indication of possible potential hazards to consumers

(Borch *et al.*, 1996). Also, five species of such bacteria have associated with food poisoning as *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis* (intoxication-diarrheal type), *Bacillus brevis* and *Bacillus sphericus* (Hadlok, 1983 and Soliman, 2013).

Relatively, little is known about the incidence of aerobic spore forming bacteria in meat and meat products. Therefore, this study was conducted to throw out light on this group of bacteria with special reference to the incidence, level of contamination and significance of such serious bacteria on the public health hazard.

2. MATERIAL AND METHODS:

2.1. Collection of samples:

A grand total of 100 random samples of meat products include frozen rice kofta, kobiba-shami, oriental sausage and beef luncheon (25 for each) were collected from different shops, supermarkets and hypermarkets in different localities in Menoufia and Kalyobia governorates. All samples were kept in an ice box during transportation to the laboratory with minimum time of delay and analyzed as rapidly as possible for presence of aerobic spore formers.

2.2. Preparation of samples:

Ten grams of each sample were taken under aseptic condition and put into a sterile blender jar containing 90 ml of 0.1% sterile peptone water and homogenized for sufficient time to give a final dilution of 1/10. Directly after maceration, 5 ml of the previously prepared 1/10 dilution were heated to 80°C in thermally controlled water bath for 10 minutes and cooled (Harrigan and McCane, 1976).

2.3. Determination of total aerobic spore formers counts:

It was carried according to Oxoid (1990) and Harrigan and McCane (1976).

2.4. Isolation and Identification of isolated aerobic spore formers:

Suspected colonies of aerobic spore formers were picked up and seeded into nutrient agar slopes then incubated at 37°C for 24 hours. The culture was morphologically and biochemically identified according to Kring and Holt (1986) and BAM (1998).

3. RESULTS:

Table (1) reported that the incidence of aerobic spore formers in frozen rice kofta, kobiba-shami, oriental sausage and beef luncheon samples were 88%, 84%, 76% & 72% and 84%, 76%, 76% at 32°C and 56% at 55°C, respectively .

The differences between the examined samples of meat products were significant ($P<0.05$) as a result of total aerobic spore formers counts at 32°C and 55°C.

Results given in table (2) showed that the total aerobic spore former counts in the examined samples ranged from 4.9×10^2 to 2.4×10^3, 1.3×10^2 to 2.2×10^3, 1.3×10^2 to 2.1×10^3 and 1.1×10^2 to 1.4×104 cfu/gm with mean values of $1.37\times10^3\pm0.29\times10^3$, $1.16\times10^3\pm0.25\times10^3$, $1.03\times102\pm0.17\times10^3$ and $8.58\times10\pm1.62\times104$ cfu/gm at 32°C (mesophilic type) and 7.0×10 to 9.5×10^2, 1.0×10^2 to 1.2×10^3, 5.0×10 to 9.2×10^3 and 4.0×10 to 5.8×10^2 with mean values of $6.14\times10^2\pm0.88\times10^2$, $6.52\times10^2\pm1.07\times10^3$, $4.79\times10^2\pm0.54\times10^2$ and $2.96\times10^2\pm0.31\times10^2$ cfu/gm at 55°C (thermophilic type) in frozen rice kofta, kobeba-shami, oriental sausage and beef luncheon, respectively.

Regarding the results in table (3), the incidence of isolated spore formers strains at 32°C and 55°C were *B. cereus* (52%,28%), *B. circulans* (8%,4%), *B. coagulans* (16%,12%), *B. licheniformis* (28%,8%), *B. macerans* (16% at 32°C) , *B. megaterium* (24% at 32°C), *B. polymyxa* (4% at 32°C) and *B. subtilis* (32%,12%), *B. pulvifaciens* 4% at 55°C), *B.*

Table (1): Incidence of aerobic spore formers in the examined meat products samples (n=25)

Meat products	At 32°C		At 55°C	
	No.	%	No.	%
Frozen Rice Kofta	22	88	21	84
Kobeba shami	21	84	19	76
Oriental Sausage	19	76	19	76
Beef Luncheon	18	72	14	56
Total (100)	80	80	73	73

Table (2): Statistical analytical results of total aerobic spore former counts/gm in the examined meat product samples (n=25).

Meat products	Temp.	Min.	Max.	Mean ± S.E*
Rice Kofta	At 32°C	102×4.9	103×2.4	103+×103± 0.29×1.37
	At 55°C	10×7.0	102×9.5	102 +×102± 0.88×6.14
Kobeba shami	At 32°C	102×1.3	103×2.2	103+×103± 0.25×1.16
	At 55°C	102×1.0	103×1.2	103+×102± 1.07×6.52
Oriental Sausage	At 32°C	102×1.3	103×2.1	103+×103± 0.17×1.03
	At 55°C	10×5.0	103×9.2	102+×102± 0.54×4.79
Beef Luncheon	At 32°C	102×1.1	103×1.4	104+×102± 1.62×8.58
	At 55°C	10×4.0	102×5.8	102+×102± 0.31×2.96

S.E* = standard error of mean
+ = Significant differences between products (P<0.05)

Table (3): Incidence of *Bacillus* species isolated from the examined meat products samples (n=25).

Meat products / *Bacillus spp.*	Frozen Rice kofta				kobeba				sausage				luncheon			
	Incubation temperature				Incubation temperature				Incubation temperature				Incubation temperature			
	At 32°C		At 55°C		At 32°C		At 55°C		At 32°C		At 55°C		At 32°C		At 55°C	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
B. cereus	13	52	7	28	11	44	6	24	8	32	5	20	5	20	2	20
B. circulans	2	8	1	4	5	20	-	-	1	4	-	-	-	-	-	-
B. coagulans	4	16	3	12	1	4	2	8	4	16	1	4	1	4	-	-
B. licheniformis	7	28	2	8	3	12	5	20	2	8	2	8	2	8	1	4
B. macerans	4	16	-	-	3	12	4	16	6	24	-	-	2	8	-	-
B. megaterium	6	24	-	-	7	24	-	-	2	8	2	8	1	4	3	12
B. polymyxa	1	4	-	-	1	4	-	-	3	12	-	-	-	-	-	-
B. pulvifaciens	-	-	1	4	1	4	-	-	-	-	2	8	-	-	-	-
B. stearothermophilus	-	-	5	20	-	-	2	8	-	-	6	24	-	-	4	16
B. subtilis	8	32	3	12	6	24	4	16	9	36	7	28	3	12	1	4
B. sphaericus	-	-	-	-	-	-	-	-	-	-	3	12	-	-	1	4

In kobeba-shami, the incidence of *B. cereus* was (44%, 24%), *B. circulans* (20% at 32°C), *B. coagulans* (4%, 8%), *B. licheniformis* (12%, 20%) *B. macerans* (12%, 16%), *B. megaterium* (24% at 55°C), *B. polymyxa* (4% at 32°C), *B. pulvifaciens* (4% at 32°C), *B. stearothermophilus* (8% at 55°C) and *B. subtilis* (24%,16%). In sausage incidence of *B. cereus* was (32%,20%), *B. circulans* (4% at 32°C), *B. coagulans* (16%,4%), *B. licheniformis* (8%,8%), *B. macerans* (24% at 32°C), *B. megaterium* (8%, 8%), *B. polymyxa* (12% at 32°C), *B. pulvifaciens* (8% at 55°C), *B. spharicus* (12% at 55°C), *B. stearothermophilus* (24% at 55°C) and *B. subtilis* (36%, 28%).

The incidence of *Bacillus* species in luncheon samples was *B. cereus* (20%, 20%), *B. coagulans* (4% at 32°C), *B. licheniformis* (8%, 4%), *B. macerans* (8% at 32°C), *B. megaterium* (4%, 12%), *B. spharicus* (16% at 55°C), *B. stearothermophilus* (4% at 55°C) and *B. subtilis* (12%, 4%).

4. DISCUSSION:

Literature extending over many years points out that meat and its products are liable to contamination with various kinds of microorganisms from different sources. Meat contamination may cause public health hazard to consumers as well as meat

handlers or impair the utility of meat especially in countries in which hygienic measures are still under way. Realizing that the demonstration of bacterial density of meat and meat products can give some indication about the hygienic quality of the products under investigation (Soliman, 2013).

The results given in table (1) reflected that the highest bacterial percentage was in frozen rice kofta followed by kobeba–shami then oriental sausage and finally beef luncheon. This may be attributed to the soaked crushed rice added to the rice kofta products which is the main source for its contamination and also spices may be the other source of contamination (Ahmed, 2002).

Moreover, the lack of sanitary measures during processing, handling and storage may act as the main source of food contamination with aerobic spore formers (Torky, 2004). These results are nearly similar to those obtained by (Soliman, 2013) who found that the incidence of aerobic spore formers in the examined luncheon, hot dog and frankfurter samples was 94%, 85.7% and 82.8%, respectively.

The achieved results in table (2) come in accordance with those obtained by Khalifa, (1997) who found that the total aerobic spore formers count at 32°C was 4x102 to 3x104 cfu/g in oriental sausage. While lower than finding those obtained by Nassif (1996) "2.54 X 105 cfu/g" and Abosrea-Nadia (2005) "6×105 cfu/g ".

Furthermore, the aerobic spore formers counts at 32°C in examined samples of beef luncheon agree with results obtained by Nassif (1996) "8 X 105 cfu/g " and Abosrea-Nadia (2005) "2×104 cfu/g ".

Results given in table (1) indicated that the highest incidence of aerobic spore formers at 55°C was in frozen rice kofta followed by kobeba-shami and sausage then luncheon. This may be due to heat treatment of beef luncheon that may have effect on this bacteria.

Table (2) reflected that the aerobic spore formers counts at 55°C in oriental sausage samples were lower than those obtained by Nassif (1996) "6.4 X 104 cfu/g".

However, the total aerobic spore formers counts at 55°C in the examined beef luncheon samples were lower those obtained by Khalifa (1997) whose result was $1.63x10^2 \pm 2.82x10^2$ and Abosrea-Nadia (2005) "5Xl03 cfu/g ".

The presence of high count of aerobic spore forming in frozen rice kofta may be attributed to the soaked crushed rice that added to the products and spices (Ahmed, 2002).

The presence of high count of aerobic spore forming in kobeba- shami may be contributed to contaminate coming from crushed wheat, spices and vegetables used in manufacture of such product as well as other bulky additives like soya (Abdallah, 2005).

The presence of high count of aerobic spore forming in examined oriental sausage may be attributed to the high content of curing salts and spices in addition to all problems of fluctuation of temperature during cooking.

The bacterial load of the examined beef luncheon samples may be attributed to several reasons as cross-contamination during processing. Also the unsanitary hygienic condition during handling, storage, transportation and marketing play a major role in bacterial contamination.

Low counts of total aerobic spore formers in the examined luncheon samples may be due to heat treatment to this product (Youssif, 1982).

Table (3) showed, the isolated aerobic spore formers microorganisms in the examined meat products samples.

Bacillus cereus in frozen rice kofta at 32°C agreed with those reported by Abd-allah (2005) who isolate *B. cereus* from 52% from frozen rice kofta samples.

In kobeba-shami, the incidence of *B. cereus* at 32°C is nearly similar to that obtained by Abd-Allah (2005) who isolated *B. cereus* from 60% from kobeba-shami samples.

In sausage, the incidence of *B. cereus*, *B. subtilis* and *B. lichiniformis* at 32°C is nearly similar to those reported by Abosrea-Nadia (2005) as incidence of them was 84%, 8% and 4%, respectively. At 55°C, the results of the incidence of *B. cereus* in sausage is nearly similar to the results obtained by Nassif, (1996) who found that its incidence was 18%, El-mosalami (2003), Amin (1995) and Hefnawey *et al.* (1984), but lower than that obtained by Eldaly (1988) who found *B. cereus* in 60% of examined sausage samples, Elsayed-Sherin (2010), Ali (1987), Lotfi *et al.* (1988) and Khalil (1997) and higher than Konoma *et al.* (1988), Shinagawa *et al.* (1985) and Shinagawa *et al.* (1984) who isolated *B. cereus* from 12% from examined sausage samples.

Bacillus cereus incidences in luncheon agree with results obtained by Samir et al. (2012) who isolated it from 20% from examined luncheon samples, but relatively lower than the results obtained by Nassif (1996) who isolated *B. cereus* from 50% of examined luncheon samples, Ali (1987), Khalil (1997), Lotfi *et al*. (1988) and Mervat *et al.* (2006), but more than results obtained by El-khawas (2001) who examined samples of luncheon for aerobic spore formers and could not isolate *B. cereus* but the incidence *B. subtilis* and *B. licheniformis* agreed with those reported by Abosrea-Nadia (2005) who found the incidence of *B. cereus*, *B. subtilis*, *B. lichiniformis* and *B. brevis* in luncheon was 80%, 2%, 4% and 4%, respectively and isolated *B. brevis* from luncheon with an incidence of 4% .

The result of *B. stearothermophilus* in beef luncheon agreed with those obtained by (Nassif, 1996) whose result was 15.8%, but lower than the incidence of *B. subtilis* (44.2%).

Aerobic spore-formers such as *B. licheniformis, B. subtilis, B. alvi* and *B. cereus* are responsible for intoxication (diarrheal type) lead to 19 cases of food poisoning (Hadlok, 1983).

Therefore, all the above-mentioned results suggested that problems associated with consumption of foodstuffs and those existing food borne disease surveillance system must receive attention and upgrading.

5. EFERENCES

Abd-Allah, A.M.S. 2005. Incidence of *Bacillus cereus* in some meat products Veterinary medicine, Moshtohor / Food control Zagazig. Benha branch.

Abosrea, Nadia, A. 2005. Bacteriological quality attributes of some meat products with special reference to aerobic spore formers and *Bacillus cereus*. J. Fac. Vet. Med., 23:77-85.

Ahmed, Dalia, M.S. 2002. Hygiene evaluation of camel meat. Ph.D., Thesis (Meat Hygiene), Fac. Vet. Med. Zagazig Univ.

Ali, G.M.1987. Incidence of *Bacillus cereus* in meat products. M.V.Sc., Thesis (Meat Hygiene, Fac. Vet. Med. Assuit Univ.

Amin, A.S.A.T.1995. Bacterio-toxicological studies of *Bacillus cereus* in meat products. M.V.Sc., Thesis, Dept. Food Hygiene, Fac. Vet Med. Cairo University.

Bacteriological and Analytical Manual on line (BAM) 1998. U.S. Food and Drug Administration. Center for Food Safty and Applied Nutrition.

Borch, E., Kant-Muermans, M. L., Blixt, Y. 1996. Bacterial spoilage of meat and cured meat products. Int. J. Food Microbiol., 33: 103-120.

El said, Sherin, A. 2010. Psychrophilic microorganisms in frozen meat products. M.V.Sc., Thesis, Dept. Food Control, Fac. Vet. Med. Zagazig Univ.

El-Daly, E., Saleh, E., Abd El-Galil, Y. 1988. Prevalence of *Bacillus cereus* in some meat products. Bull. Fac. Sci. Zagazig Univ., 1:25-30.

EL-Khawas, K. M. S.2001. Studies on vacuum meat products. Ph. D., Thesis (Meat Hygiene), Fac. Vet. Med.

Moshtohour, Zagazig Univ., Benha Branch.

El-Mossalami, Eman, I.K. 2003. Risk assessment of ready prepared meat products. Ph.D., Thesis (Meat Hygiene), Fac. Vet. Med., Cairo Univ.

Galvez, A., Abriouel, H., Benomar, N., Lucas, R. 2010. Microbial antagonists to food-borne pathogens and biocontrol. Current Opinion in Biotechnol., 21: 142-148.

Hadlok, R.M.1983. Die bedeutung der gattung *Baciilus* in der lebensmittlehygieneschriftenreile. Schmeiz Ges. Lebensmittlhygiene, 13:68.

Hafnawy, Y.A., Youssif, H., Abdel-Rahman, H., Lotfi, A.1984. Occurrence of *Bacillus cereus* in selected meat products. Fleischwirtschaft, 64(11):1371-1372:1394-1395.

Harrigan, W.F., McCance, M.E.1976. Laboratory Methods in Food and Dairy Microbiology. Revised Edition. Academic Press, London and New York.

Khalifa, E.M.I.1997. Aerobic spore formers in some locally manufactured meat products, Fac. Vet. Med., Dept. Meat Hygiene.

Khalil, B.G. 1997. Incidence of *Bacillus cereus* in some food stuffs with special reference to its production of thermonuclease enzyme in Assuit city. Assuit Vet.Med. J., 75:55-63.

Konoma, H., Shinagawa, K., Tokumaru, M., Onoue, K., Konno, S., Fujino, N., Shigehisa, T., Kurata, H., Kuwabara, Y. and Curlos, A.M. 1988. Occurrence of *Bacillus cereus* in meat products, raw meat and meat product additives. J. Food Protec., 51(4):324-326.

Kring, N.T., Holt, J.G.1986. Bergey`s Manual of Systematic Bacteriology. (2), Williams and Wilkins, Baltimore, London.

Lotfi, A., Youssef, H., El-Khateib, T., Seddik, L., Elimawry, A., Ali 1988. Incidence of *Bacillus cereus* in meat products. Assiut Vet. Med. J., 39:111-114.

Mervat, A.M.A., Dalia, A., Hala, N.E.l., Hifnawi. 2006. Incidence of *Bacillus cereus* in some meat products and the effect of Gamma radiation on its toxins. Int. J. Agric. And Biol., 04-1-1-08/8530-1560.

Nassif, M.R.M.1996. Occurrence and significance of aerobic spore forming microorganisms in some meat products with special reference To *Bacillus cereus*. Ph..D., Thesis, Food Control Dept.. Fac. Vet. Med. Suez-Canal Univ.

Oxoid Manual.1990. Culture media, ingredients and other laboratory services. 6th Ed. Oxoid Ltd., London.

Samir, M.M., Hanan, M.T.E., Wafa, F.A.2012. Incidence of *Bacillus cereus* in some raw and cooked meat products and its control by heat treatment. Proceedings of the 5th Scientific conference of animal Wealth Research in the Middle East and North Africa, Faculty of Agriculture, Cairo University, Giza, Egypt, 1-3 October: 182-190 .

Sharma, D., Sharma, V.D., Kumar, A.1996. Microbial quality of commercial pork products. Indian J. Animal Sci., 66:21 1.

Shinagawa, K., Konuma, H., Kurata, H., Tanabayachi, K., Masusaka, N.1984. Surveillance of raw meat, meat products and meat product additives for contamination with *Bacillus cereus* and enterotoxigenisty of the isolated strains. J. Fac. Agric. Iwate. Univ., 17 (2): 175-182.

Shinagawa, K., Konuma, H., Kurata, H., Tanbyashik, K., Mastsusaka, N.1985. Surveillance of raw meat products and meat product additives for contamination with *Bacillus cereus* and enterotoxigenicity of the isolated strains. Fac. Agric. Iwate Univer., Moriofea, Japan.

Soliman, D.H.M. 2013. Aerobic spore formers in some meat products.

M.V.Sc., Thesis (Meat Hygiene), Fac. Vet. Med., Alexandria Univ.

Torky, Amal, A.S. 2004. Trials for inhibition of some food poisoning microorganism in meat products. Ph.D., Thesis (Meat Hygiene), Fac., Vet Med., Cairo Univ.

Youssef, H.1982. Problems occurrence of putrefactive anaerobes in some meat products. Assuit Vet. Med. J., 9:97.

الميكروبات المتجرثمة الهوائيه فى بعض منتجات اللحوم

همت مصطفى ابراهيم[1]، امانى محمد سالم[1]، داليا عاطف سالم[2]، غادة عبد الحكيم عبد الحميد[2]

[1] قسم مراقبة اغذية -جامعة بنها – كلية الطب البيطري بمشتهر، [2] معهد بحوث صحة الحيوان معمل فرع شبين الكوم

الملخص العربي

أجريت هذه الدراسة على عدد 100 عينة من منتجات اللحوم وهى كفتة أرز مجمدة و كبيبة شامي و سجق ولانشون بقرى (25 من كل منتج) والتي تم جمعها عشوائيا من المحلات والسوبر ماركت فى نطاق محافظتي المنوفية والقليوبية هذا وقد أسفرت نتائج الاختبارات البكتيريولوجية على الآتي: نسبة تواجد الميكروبات الهوائية المتحوصلة هى 88%، 84%، 76%، 72% وبمتوسط العد الكلى لهذه الميكروبات هو 1,37×310±0,29×310، 1,16×310±0,25×310 1,03×310±0,17×310، 8,58×210±1,62×410 لكل من كفتة أرز مجمدة و كبيبة شامي و سجق ولانشون البقرى على الترتيب عند درجة حرارة 32°م . أما نسبة تواجدها عند درجة حرارة 55 °م كانت 84% ، 76% ، 76% ، 72% وبمتوسط العد الكلى 6,14 ×210±0,88×210، 6,52 ×210±1,07×310، 4,79 ×210±0,54×210، 2,96×210±0,31×210 على التوالي . وقد تم عزل وتصنيف الميكروبات الهوائية المتحوصلة وكانت نسبة الباسيلس سيريس أعلى نسبة تلوث في تلك المنتجات. وهذا وقد تم مناقشة الأهمية الصحية لهذه الميكروبات المعزولة ومدى تأثيرها على المنتج وصحة المستهلك وقد تم مناقشة الإجراءات الصحية الواجب اتخاذها أثناء تجهيز وتصنيع تلك المنتجات لتقليل التلوث بها للحصول على منتج نهائي صحي ذو كفاءة عالية لا يشكل خطر ولا يؤثر على صحة المستهلك.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26 (2)، يونيو 2014: 219-226)