Plant Archives Vol. 20 Supplement 1, 2020 pp. 3002-3006 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BACTERIOLOGICAL ASSESSMENT FOR CLEANING AND SANITIZING OF DOMESTIC MILKING EQUIPMENT BY USING OZONATED WATER

Zina saab Khudhir

Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract

A total of 20 raw milk samples were used as the fouling agent for evaluating the bacteriological effectiveness of cleaning and sanitizing of domestic milking equipment by using ozonated water at 0.5 ppm comparing to the warm water at 55! for 5 minutes respectively. The mean values of total aerobic bacteria, Coliform and *E.coli* that present on the plastic and stainless-steel containers after using the raw milk as fouling agent were 3.4×10^{-6} , $6.7x10^{-5}$ and 5.8×10^{-3} cfu/cm^2 respectively , after cleaning the stainless steel containers by the ozonated water the mean values of total aerobic bacterial counts, Coliforms and *E.coli* bacteria were reduced to 1.2×10^{-6}, 4.7×10^{-5} and 3.3×10^{-3} CFU/cm^2 respectively. while after cleaning by the warm water were only reduced to $2.3x10^{-6}$, $2.7\times10^{-5}$and 2.8×10^{-3} CFU/cm^2 respectively . The mean values of total aerobic bacteria, coliform ,and *E.coli* counts/ cm^2 after cleaning the plastic containers by the ozonated water were reduced to 1.4×10^{-6}, 1.8×10^{-5} and 2.4×10^{-3} CFU/cm^2 respectively, while the mean values after cleaning by the warm water were reduced to 1.1×10^{-6}, 1.7×10^{-5} and 2.3×10^{-3} CFU/cm^2 respectively .The mean values of total aerobic, Coliforms and *E.coli* bacteria after using both the Ozonating and warm water for both stainless steel and plastic containers were reduced to $4.2x10^{-5}$, $2.1x10^{-3}$, $1.6x10^{-2}$, $2.2x10^{-5}$, $5.1x10^{-3}$ and $1.4x10^{-2}$ CFU/cm^2 respectively. The results of the current study showed that the independed either treatment by the ozonated or warm water not effectively reduced the number of the quality control bacteria because such reduction in bacterial population could not be enough to guarantee the safety of the home dairy containers, while the combined both treatments had a significant reduction in the population of the quality control bacteria. The effectiveness of cleaning and sanitization by the ozonated and warm water process may be depending on the initial count contaminated bacteria of raw milk, exposure time, kind of containers and both concentration of ozone and the heating temperature.

Key words: ozone treatment, cleaning, sanitizing, domestic dairy equipment.

Introduction

Surfaces of domestic dairy equipment such as open stainless steel and plastic containers can subject to the different kinds of contaminations by different types of microorganisms due to the accumulation of the food raw material which is defined as Fouling, (Mengyuan, 2014) .Improper of cleaning and sanitizing of food contact surfaces and cross contamination are important factors that are responsible for the foodborne illnesses therefore many of foodborne diseases can be prevented by using a suitable surfaces cleaning and sanitizing process . Cleaning process is defined as the complete removal of food residues by using the appropriate detergent while Sanitizing process from the public health viewpoint is defined as the diminish of many kinds of microorganisms to the level which is considered as safe (Tiwari and Rice., 2012). Ozonation methods with appropriate ozone concentration and exposure time have been legally approved in the food processing in the many of European countries such as North America, Australia, and Japan (O'Donnell., *et al.,* 2013). The cleaning and disinfection processes such as hot water and chemical, detergents are usually used in the many small and large dairy plants but these processes consuming energy, chemicals and huge amount of water therefore the use of ozonation method can diminish the loss of energy that need for preparation of hot water and also diminish the high chemical costs (National Restaurant Association Educational Foundation (Nraef., 2005). Milk stones are a stronger deposit-surface that formed during the heating and pasteurization treatment with difficulty to remove this kind of deposit from the container surfaces (Liu *et al*., 2006). Milk is a highly perishable food that is usually in contact with different kinds of containers such as stainless steel and plastic surfaces during the home processing ,handling and storage stages (Barnes *et al*., 1999). Hot water can be used during the cleaning of domestic dairy equipment where Kulkarni *et al.,* (1975) reported that cold water (10-15)! or hot water (75- 80)! water has the similar activity for removal the proteineous stones from the dairy equipment. The aim of the current study was for evaluating the bacteriological effectiveness of the ozonation treatment (aqueous) at concentration of 0.5 ppm for 5 minutes compared to the warm water at 55°C for 5 minutes for reducing the bacterial counts on surfaces of both open plastic and stainless-steel containers after spoilage them by raw milk for 10 minutes as residence time (exposure time).

Materials and Methods

Three liters for each of twenty raw milk samples were used as the fouling agent for evaluation each of the warm water effectiveness at (55°)! for 5 minutes and ozonized water at 0.5 ppm for 5 minutes as cleaning and sanitizing agents for the domestic plastic and stainless steel containers. The cooled raw milk samples were poured into both open plastic and stainless steel containers with the contacting times 10 minutes at ambient temperature. A total of 20 raw milk samples were purchased from the local markets in Baghdad city. The domestic milking utensils that used in the current study were properly cleaned , disinfected and dried before each treatment. The milk samples were immediately transferred to the veterinary medicine lab in the cooled polyethylene bags for the bacteriological analysis.

Ozonation treatment

The apparatus that used for generation of ozone was (A2Z/AQUA-6) with diffuser rate at 600 mg/h and the dose of ozone was 0.6 ppm. The calculation of the ozone concentration (ppm/in water) was done by using the CHE-Mets-Kit which was used for the measurement of the highest ozone concentration between the time that used (5 minutes) and the ozone concentration (0.5 ppm) where the aeration stone was inserted into in the sterile distal water in both plastic and stainless steel containers at ambient temperature (30^0C).

Containers surfaces sanitation before treatment:

Prior pouring the raw milk samples all the containers were washed carefully by sterile distal water and sanitized by using ethanol at 70% (v/v) for 2 minutes (Botti *et al*., 2014). All the complete processes of container spoiling, dilution techniques and culturing methods were performed in a U-V laminar flow cabinet to avoid environmental contamination. The improvement of the homogeneity of milk distribution on the surfaces of domestic containers continuously occurred by manual agitating for 3 minutes to guarantee the complete soiling of containers surfaces and the containers placed inside the U-V laminar flow hood for 10 minutes (contact time), before the bacteriological examination.

Bacteriological analysis

All the containers were filled with warm and ozonated water at concentrations and the exposure times as mentioned above then the surfaces of the home open plastic and stainless steel containers were bacteriological examined by using the cotton swab technique which pre-wetted in the sterile nutrient broth at the area of 50 cm^2 horizontally. The surfaces of domestic milking containers were randomly selected and the cotton swabs were immersed in the sterile nutrient broth for five minutes, mixed well and then the average of total aerobic bacteria, coliforms and *E.coli* counts were recorded as the colony forming units (CFU) per 50 cm^2, tenfold serial dilutions from 10^{-1}to10^{-7} were prepared in the sterile 0.1% (wt/v) peptone water then plated in the Petrifilm, (3M™ St. Paul, MN) for aerobic bacterial counts, Coliform and *E. coli* counts which were used following the manufacturer's instructions (Fig. 1). The petrifilm Plates were incubated at 35°C±2! for 24 to 48 hours then the plates were counted by colony counter. Three replicates of the cotton swabs technique were taken and the average values were calculated before and after each of warm and ozonated water treatments.

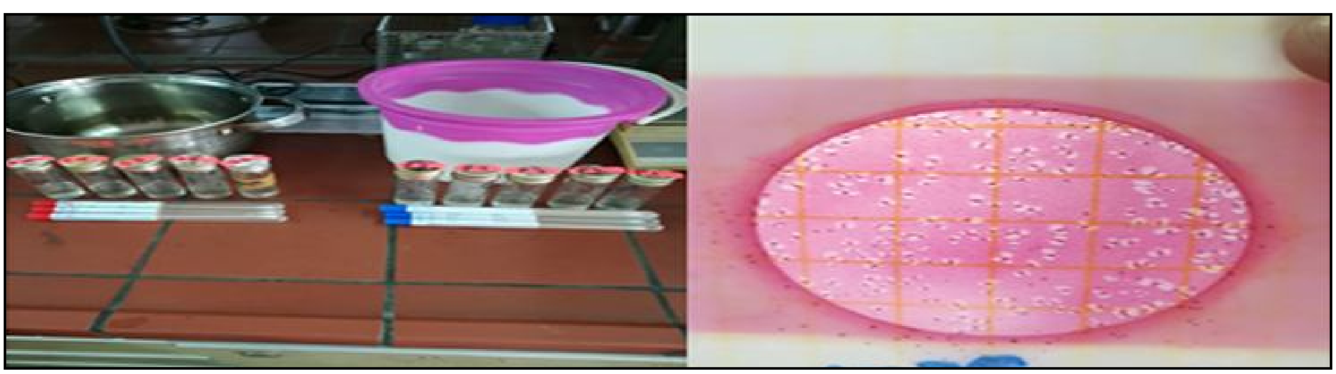

Fig. 1: Isolation Coliform bacteria by Petrifilm, (3M™ St) from domestic open surfaces of plastic and stainless- steel containers.

Statistical analysis:

The current data were calculated statistically by using ANOVA and determined the differences between results by Tukey test, with confidence level of ($p<0.05$) (Statistical Analysis System SAS/STAT, user's guide for Personal computer.

Result and Discussion

Results of the cultural properties of the quality control bacteria such astotal aerobic bacteria, coliforms and *E.coli* are presented in the Table 1. The Petrifilm, (3M™ St. Paul, MN) for aerobic Counts , Coliform and *E. coli* counts were used following the manufacturer's instructions.

The initial bacterial counts for total aerobic bacteria, coliforms and *E.coli* that isolated from stainless-steel container surfaces after using raw milk as fouling agent were $3.4x10^{-6}$, $6.7x10^{-5}$ and $5.8x10^{-3}$ respectively table 2. For all the microorganisms that determined in the current study bacterial analysis were repeated triplicate. The bacterial population on the surfaces of either open plastic or stainless steel containers after pouring the raw milk declined as the response of rinsing by both warm and ozoneted water at (55°C) and 0.5 ppm for 5 minutes respectively. ozonated water treatment at 0.5 ppm for 5 minutes resulted in a reduction in the bacterial contaminations significantly higher than the warm water at 55 degrees ! for 5 minutes. The mean values of total aerobic bacteria, Coliform and *E.coli* that isolated from plastic and stainless-steel container surfaces after using raw milk as fouling agent were $3.4x10^{-6}$, $6.7x10^{-5}$ and $5.8x10^{-3}$ cfu/cm^2 respectively Table 2, when the home stainless steel containers cleaned by ozonated water the mean values of total aerobic bacterial counts ,Coliforms and *E.coli* bacteria were $1.2x10^{-6} \pm 0.693$, $4.7\times10^{-5} \pm 1.714$ and $3.3x10^{-3} \pm 1.905$ CFU/cm^2 respectively. While the mean values after cleaning treatment by warm water were $2.3x10^{-6} \pm 1.328$, $2.7\times10^{-5} \pm 1.559$ and $2.8\times10^{-3} \pm 1.617$CFU/cm^2 respectively as shown in Table 3. The mean values of the total aerobic bacteria, coliform and *E.coli* counts/cm^2 after cleaning the open home plastic containers by ozonated water were $1.4\times10^{-6} \pm 0.866$, $1.8 \times 10^{-5} \pm 1.039$ and $2.4 \times 10^{-3} \pm 1.097$ CFU/cm^2 respectively, while the mean values after cleaning by warm water were $1.1 \times 10^{-6} \pm 0.635$, $1.7\times10^{-5} \pm 0.866$ and $2.3\times10^{-3} \pm 1.328$ CFU/cm^2 respectively Table 4. The mean values of total aerobic, Coliforms and *E.coli* bacteria after using both Ozonation and warm water cleaning for stainless - steel and plastic containers were $4.2\times10^{-5} \pm 2.425$, $2.1\times10^{-3} \pm 1.212$,$1.6\times10^{-2} \pm 0.924$, $2.2\times10^{-5} \pm 1.212$,$5.1\times10^{-3} \pm 2.887$ and $1.4\times10^{-2} \pm 0.808$ CFU/cm^2 respectively Table 4. The in dependent treatment by either ozonated or warm water not effectively reduced the number of quality control bacteria to be enough to guarantee the safety of home dairy containers, while the combined both treatments had a significant reduction in the population of quality control bacteria by 1-2 log cycle. The effectiveness of cleaning and sanitization by ozonated and warm water processes may be depending on the initial contamination, contact time and kind of containers.

Cleaning practices during the milking process is very important for the production of good keeping quality milk and dairy products , many kinds of bacteria and dirt can be transported from the animal udder and teats ,farmers hands and milk containers that are used during the milking process and transportation as many of milkers and farmers used plastic jars for milking equipment and usually these home plastic containers are not food–grade may cause the health hazard (Fufa Abunna *et al.,* 2019). While the aluminum cans are more recommended because they are easily to clean than occur with plastic containers because the aluminum and stainless-steel containers don't have the adhesive characterizes (Fufa Abunna *et al.,* 2019). The Most common cans or containers that are used in the home made farmers , small producers in the dairy farm are plastic and stainless steel containers with volume of 3 to 5 liters while the plastic containers are characterized by the adhesive properties with difficultly to clean (Kasirayi Gwezuva., 2011). The results obtained that presented in (Table 2) clearly indicated that the bacterial counts recovered from the surfaces of the plastic and stainless-steel containers (Mean log 10cfu/cm^2) after pouring raw milk (soiling) before cleaning treatments for total aerobic bacteria, coliform and *E.coli* counts were

Table 1: The Cultural characteristics of the quality control bacteria that used in the current study.

Quality control bacteria	Media	Cultural properties
Total Aerobic bacteria	Petrifilm, (3M™ St)	Red colonies or pink colonies
Coliform bacteria	Petrifilm, (3M™ St)	Red colonies with gas
E.coli bacteria	Petrifilm, (3M™ St)	Blue colonies with gas

Total aerobic, Coliform and *Ecoli* counts detected in each domestic containers are shown in Table 2. The milk samples that were positive for total aerobic bacteria, coliform and *E.coli* bacteria were (20/20) and (19/20) respectively. The bacterial counts recovered from the surfaces of the plastic and stainless steel containers (Mean log 10cfu/cm^2) after pouring raw milk (soiling) before treatments demonstrated that the total aerobic bacteria, coliform and *E.coli* counts were $3.4x10^{-6}$, $6.7x10^{-5}$ and $5.8x10^{-3}$ cfu/ml respectively.

3.4×10^{-6}, 6.7×10^{-5} and 5.8×10^{-3} cfu/cm^2) respectively. The overall bacterial counts for total aerobic bacteria, coliform and *E. coli* were high and such high bacterial contamination levels can presented the potential public health hazard due to growth and multiplication of different kinds of spoilage and pathogenic microorganisms and such high contamination level can be contributed to the unhygienic processes during all the stages of milk collection and transportation by open dairy containers especially in the rural area surrounding the Bagdad city . The ozonated water treatment at 0.5 ppm for 5 minutes resulted in reduction in the bacterial contaminations significantly higher than the warm water at 55 degrees ! for 5 minutes such data come in parallel with the study of Jindal *et al*., (1995) who concluded that the ozone treatment reduced the contamination by the aerobic bacterial counts, coliforms and *E. coli* bacteria on the food surfaces as in drum stick by more than one log cycle with the shelf-life extension much as two days (Hanan *et al*., 2013). The current study showed that using ozonated or warm water alone not effectively reduced the number of quality control bacteria as table 3 and 4 as such reduction in the bacterial population could not be enough to guarantee the safety of home dairy containers. The first step in the cleaning of the dairy utensils is rinsing with warm water at (40°C), this step is important for removing soil and dirt's from the milking equipment, the surfaces materials assessment done by direct surface sampling which is regarded as the real indication of the efficiency of cleaning process (Mengyuan..Fan, 2014). The conventional swabbing technique is the recommended technique for cleaning practices for many types of material surfaces as wood, plastic and stainless- steel (Rached Ismaïl *et al.,* 2013). Bacteriological evaluation of ozonated water and warm water treatments at ambient (30!) temperature on the stainless-steel container indicated that the ozonated water was more effective than warm water on the bacterial contamination present on the surfaces of stainless-steel container rather than plastic containers which may be attributed to the nature of the plastic cans material with the hydrophobic properties that exhibiting greater bacterial surfaces adherence comparing with aluminum containers, glasses and stainless steel with hydrophilic properties materials (Wanjala Nobert Wafula *et al.,* 2016). The potent antimicrobial properties, broad spectrum activity and very effective oxidation capacity of ozone even at relatively low concentrations level, without any residual effect also the easily of stain steel surfaces to cleaning processing Ozone has antibacterial activity against viruses and both

Table 2: Number of positive isolates and bacterial counts recovered from surfaces of the plastic and stainless- steel containers (Mean log 10cfu/cm2) after pouring raw milk (soiling) before treatments.

Number of raw milk samples	**Positive and counts of quality control bacteria (Mean log $_{10}$cfu/cm^2)**					
	Total aerobic bacteria		Coliform bacteria		*E.coli* bacteria	
	NO of positive samples	counts	NO of positive samples	counts	NO of positive samples	counts
20	20/20	3.4x10-^{6}	19/20	6.7x10-^{5}	19/20	5.8x10-^{3}

Table 3: Bacteriological evaluation of ozonated water and warm water treatments at ambient (30!) temperature on the stainless-steel container surfaces after using raw milk as fouling agent.

Quality control bacteria	**The initial bacterial counts (control)**	**ozonated waterMean log ±SE(log$_{10}$ cfu/ cm^2)**	**warm waterMean log ±SE(log$_{10}$cfu/ cm^2)**	**Both treatmentsMean log ±SE(log10 cfu/ cm^2)**
Total Aerobic bacteria	3.4x10-^{6}	1.2x10-^{6}±0.693Aa	2.3x10-^{6}±1.328Aa	4.2x10-^{5}±2.425Ab
Coliform bacteria	6.7x10-^{5}	4.7x10-^{5}±1.714 Ba	2.7x10-^{5}±1.559 Ba	2.1x10-^{3}±1.212Bb
E.coli bacteria	5.8x10-^{3}	3.3x10-^{3}±1.905Ca	2.8x10-^{3}±1.617Ca	1.6x10-^{2}±0.924Cb

Means with different capital letters in the column are significantly different (P<0.05) between the quality control bacteria
Means with different small letters in the row are significantly different (P<0.05) between different the cleaning treatments.

Table 4: Bacteriological evaluation of ozonated water and warm water treatments at ambient (30!) temperature on the plastic container surfaces after using raw milk as fouling agent.

Quality control bacteria	**The initial bacterial counts (control)**	**ozonated waterMean log ±SE(log$_{10}$ cfu/ cm^2)**	**warm waterMean log ±SE(log$_{10}$cfu/ cm^2)**	**Both treatmentsMean log ±SE(log10 cfu/ cm^2)**
Total Aerobic bacteria	3.4x10-^{6}	1.4x10-^{6}±0.866Aa	1.1x10-^{6}±0.635Aa	2.2x10-^{5}±1.212Ab
Coliform bacteria	6.7x10-^{5}	1.8x10-^{5}±1.039Ba	1.7x10-^{5}±0.866Ba	5.1x10-^{3}±2.887Bb
E.coli bacteria	5.8x10-^{3}	2.4x10-^{3}±1.097Ca	2.3x10-^{3}±1.328Ca	1.4x10-^{2}±0.808Cb

Means with different capital letters in the column are significantly different (P<0.05) between the quality control bacteria.
Means with different small letters in the row are significantly different (P<0.05) between different the cleaning treatments.

Gram- positive and Gram- negative bacteria (Marco., Remondino and Luigi ., Valdenassi., 2018). In the previous study (Khudhir and Mahdi., 2017) the results indicated that hurdle method as ozonated water and storage at refrigeration temperature can acted synergistically for minimizing the total bacterial counts, coliforms and yeasts and molds population of the local bovine and ovine soft cheese in Baghdad city. The diminished bacterial population was the most signiûcant for ozonated water followed by the warm water as illustrate in tables 3 and 4, the mean values of total aerobic, Coliforms and E.coli bacteria after using both Ozonation treatment and warm water for stainless steel and plastic containers were $4.2\times10^{-5} \pm 2.425$, $2.1\times10^{-3} \pm 1.212$, $1.6\times10^{-2} \pm 0.924$ and $2.2\times10^{-5} \pm 1.212$, $5.1\times10^{-3} \pm 2.887$ and $1.4\times10^{-2} \pm 0.808$ CFU/cm^2 respectively.

Conclusion

Using of both warm water and ozonated water synchronously in the cleaning and sanitization of domestic containers were more effective than using the individual treatment.

No ethical approval was required because no live animal was used in the study. All the raw milk samples were collected from local markets in Baghdad city.

References

Mengyuan Fan, B.S. (2014). Effectiveness of pre-rinse during in-place cleaning of stainles steel pipe lines. thesis., Ohio State University .pp 9.

Tiwari, B.K. and R.G. Rice (2012). Regulatory and legislative issues. In Ozone in Food Processing, pp 7–17.

O'Donnell, C., B.K. Tiwari, P.J. Cullen and R.G. Rice (2013). eds. Oxford: Wiley- Blackwell. Heacox D Use and generation of ozone as a disinfectant of dairy animal tissues, dairy equipment, and infrastructure. Patent No. US 8609120 B2.

National Restaurant Association Educational Foundation (NRAEF) (2005). Serve safe Essentials. The Educational Foundation of the National Restaurant Association, Chicago, IL.

Liu, W., G.K. Christian, Z. Zhang and P.J. Fryer (2006). Direct measurement of the force required to disrupt and remove fouling deposits of whey protein concentrate. *International Dairy Journal,* **16(2):** 164-172.

Barnes, L., M, M.F. Lo, M.R. Adams and A.H.L. Chamberlain (1999). Effect of milk proteins on adhesion of bacteria to stainless steel surfaces. *Applied and Environmental Microbiology,* **65(10):** 4543-8.

Kulkarni, S.M., R.G. Arnold and R.B. Maxcy (1975). Reuse imits and regeneration of solutions for cleaning dairy equipment [J]. *Journal of Dairy Science,* **58(8):** 1095-1100.

Botti, L.C.M., P.F.J. Bócoli, A.S.M.L. Leber, L.B. Iarada, A.A.L. Tribst and M. Cristianini (2014). Sanitation of plastic bottles using ozonated water, *International Food Research Journal,* **21(4):** 1375-1379.

SAS (2001). SAS/STAT, user's guide for Personal computer, Release 9, SAS. Institute. Inc. Cary. NC. USA.

Fufa Abunna, Nigus Tasew, Fikru Ragassa, Dinka Ayana and Kebede Amenu (2019). Handling Practices, Quality and Safety of Milk along the Dairy Value Chains in Selected Sub Cites of Addis Ababa, Ethiopia, *Biomedical Journal of Scientific and Technical Research,* **13(1):** 1-14.

Kasirayi Gwezuva (2011). An analysis of raw milk transportation and possible strategies to maintain milk quality in Matabeleland region, Zimbabwe. thesis, Larenstein University of Applied Sciences.

Vineet Jindal, Amyl. Waldrou and Richard H. Forsythe (1995). Ozone and improvement of quality and shelf life of poultry products, *J. Appl. Res.,* **4:** 239-249.

Hanan, A. EL dahshan, Taghreed, A. Hafaz and Hanan A. EL ghayaty (2013). Effect of Ozone on preservation of chilled chicken, *Assiut Vet. Med. J.,* **59(136):**.

Rached Ismaïl, Florence Aviat, Valérie Michel, Isabelle Le Bayon, Perrine Gay-Perret, Magdalena Kutnik and Michel Fédérighi (2013). Methods for Recovering Microorganisms from Solid Surfaces Used in the Food Industry: A Review of the Literature., *International Journal of Environmental Research and Public Health,* **10:** 6169-6183.

Wanjala Nobert Wafula, Wafula Joseph Matofari, Masani John Nduko and Peter Lamuka (2016). Effectiveness of the sanitation regimes used by dairy actors to control microbial contamination of plastic jerry cans' surfaces, *International Journal of Food Contamination*, **3(9):** 2-8.

Marco, Remondino and Luigi, Valdenassi (2018). Different Uses of Ozone: Environmental and Corporate Sustainability. Literature Review and Case Study, *Sustainability,* **10(12):** 4783.

Khudhir, Z.S. and A.M. Mahdi (2017). The Efficacy of Ozonated Water on the Microbial Quality of Locally Produced Soft Cheese in Baghdad, *International Journal of Science and Research (IJSR),* **6(10):** 338-387.