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Aims and Scope

Acta Veterinaria Eurasia (Acta Vet Eurasia) is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine and published three times a year (January, May and September). The publication language of the journal is English.

Acta Veterinaria Eurasia (Acta Vet Eurasia) aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of veterinary medicine. The journal publishes original articles, reviews, case reports, short communications, and letters to the editor that are prepared in accordance with the ethical guidelines.

The scope of the journal covers all animal species including the topics related to basic and clinical veterinary sciences, livestock breeding and husbandry, veterinary genetics, animal nutrition and nutritional diseases, zoonoses, veterinary medicinal products and public health, and food hygiene and technology.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of veterinary medicine.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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All expenses of the journal are covered by the of İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine. Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at actaveteurasia.istanbul.edu.tr. The journal guidelines, technical information, and the required forms are available on the journal's web page.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

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Instructions to Authors

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Editorial Policy

The submitted articles/materials must not be under consideration for publication anywhere else except in a limited form (e.g. abstract books of congresses or symposiums, part of MSc or PhD theses). The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice). Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization. Manuscripts submitted to Acta Veterinaria Eurasia will go through a double-blind peer-review process. Each submission will be reviewed by at least two independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The Editor in Chief is the final authority in the decision-making process for all submissions.

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Books with a Single Author: Combs, G.F., 1992. *The Vitamins: Fundamental Aspects in Nutrition and Health*. Academic Press, San Diego.

Conference Proceedings: **Cardinali, R., Rebollar P.G., Mugnai, C., Dal Bosco, A., Cuadrado, M., Castellini, C., 2008.** Pasture availability and genotype effects in rabbits: 2. development of gastrointestinal tract and immune function of the vermiform appendix. In: Proc. 9th World Rabbit Congress, Verona, Italy, 1159-1164.

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Pathogens Transmission and Cytological Composition of Cow's Milk

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Abstract

The article deals with the data on the quantitative and species composition of somatic cells in milk of cows of Black spotted breed. In the main period of lactation, the number of somatic cells in milk is up to 100 ths/cm³. In cases of subclinical mastitis, the somatic cell count in the udder secretion increases to 30-35 mL/cm³. However, it should be noted that in the case of subclinical mastitis their number increases in thousands times. Thus, studying the species composition of somatic cells and morphological structure of basophils in milk of cows with subclinical mastitis, we did not find any relationship between their number, morphological structure

and period of disease. Results of our study show that pathogenic staphylococci (*Staphylococcus aureus*) were the cause of subclinical mastitis in 67-73% of cases. *Streptococcus agalactiae* caused the disease in about 20% of all cases. The results of the study of bacterial contamination of the udder skin showed that regardless of the animal age, pathogens of subclinical mastitis are always present on the udder skin. The main carrier of the subclinical mastitis pathogens from the sick animal to the healthy one is the rubber of milking cups.

Keywords: Cow, milk, somatic cells

Introduction

Milk and dairy products make up a huge part of the food chain of people of any age. In addition to the main components (fat, protein, carbohydrates), cow milk contains about 150 nutrients (vitamins, micro-, macroelements, etc.), which are important for the vital functions of the human body. In addition to the fact that milk and dairy products are essential for life of people, they are also a good nutritional medium for the development of microorganisms. And in case of violation of the sanitary conditions of milk collecting and storing, milk can become a dangerous source of infections (Jensen and Newburg, 1995; Ma et al., 2000).

According to the World Health Organization (WHO), as well as the statistical results of the Sanitary and Epidemiological Service of Ukraine, milk and dairy products are classified in the first

category of risks that cause food intoxication of microbial etiology. At present, one of the most important conditions for the export of domestic dairy products to European markets is the achieving of European level of quality and safety according to the European Union standards. This is an extremely important and responsible task, since the problem of the dairy products safety in Ukraine has not been resolved. According to the international food standard, it is not enough to control the quality and safety of products at the final stage, since it cannot guarantee its real safety. High quality in physico-chemical composition, milk collected in unsanitary conditions can quickly become unsuitable for human consumption or harmful to health. However, high quality and safe milk can only be collected from healthy animals. To solve such problems, modern world food industry introduces new quality management systems. One of them is HACCP (Lelieveld et al., 2016; Romain et al., 2000).

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The quality of milk and milk products and its epidemiological safety, to a large extent, depends on the sanitary state of the technological equipment, inventory and containers. The reason for the release of inappropriate quality products, as a rule, is their poor quality washing and disinfection. Sanitary treatment of milking equipment and dairy equipment is a mandatory operation in the technological process of obtaining, primary processing, storage and transportation of milk. During its operation, on the surfaces in contact with milk, its residues, protein-fat deposits, milk stones gradually accumulate, which in the future is a favorable environment for the development of microorganisms. Therefore, after each milking, it is necessary to carry out sanitary treatment of the entire set of dairy equipment using highly effective detergents and disinfectants without violating their application regimes (Murphy and Boor, 2000).

Among the diseases of dairy cows, mastitis, especially its subclinical (latent) form, deserves special attention. The main cause of this disease is the violation of housing conditions and milking technologies. Non-compliance with the milking technology (violation of the vacuum condition, old rubber of milking cups, "dry milking", etc.) cause microtrauma of the skin, milk epithelium, and parenchyma of the udder. As a result, there are some negative environmental factors, which are subsequently complemented by the pathogenic microflora (Hussain et al., 2012; Olde et al., 2010).

An important indicator of milk safety is the presence of somatic cells (blood cells and epithelial cells that are rejected from the secretory part of the udder and streak canals). According to the cell theory of inflammation, under the inflammatory process in the mammary gland (mastitis) the number of leukocytes increases and the process of phagocytosis begins. As a result, the total number of somatic cells increases, which is an indicator of the cow's udder condition. At the same time, not only the number of somatic cells changes, but also the ratio of their species composition. Thus, to diagnose subclinical mastitis, cytological examination can be used (Dufour et al., 2011; Schalm et al., 1971; Wilson et al., 1997).

Literature data suggest the following changes in the milk composition from quarters definitely positive to mastitis screening tests based on somatic cell counts compared to normal quarters. Although most of the changes in milk composition in high cell count milk can be related to decreased synthesis or increased "leakage" due to damage to udder tissue, these explanations are obviously over simplified and much more complex phenomena are involved in the total changes occurring (Schukken et al., 2003; Schultz, 1977).

The second indicator of milk safety is the bacterial contamination which reflects sanitary conditions of milk production the most accurately. The number of somatic cells depends on the cow's udder condition. But the bacterial contamination depends on many factors: milking conditions, sanitary condition of the

milking equipment, cleanliness of the cow udder and skin covering adjacent to the udder, etc. (Knight-Jones et al., 2016).

So, the determination of the quantitative and species composition of somatic cells in the milk of clinically healthy animals and animals with subclinical mastitis, as well as to find out the main sources and ways of milk contamination by the microflora is relevant and requires more detailed research.

Material and Methods

The research protocol of the current study was approved by the Ethic Committee of the Sumy National Agrarian University (Approval number: 2017/01).

The work was carried out in the Laboratory of Clinical Diagnostics of the Sumy National Agrarian University and in conditions of production at the FH "Vladana" of the Sumy region (North-eastern Ukraine) during May-June of 2017.

Animals

The study was conducted on cows of Black-spotted breed (I-IV lactation).

The experiment involved 780 heads of cows, from which 4 groups of animals with evidence of subclinical mastitis were formed. First (I) group (the first lactation) included 10 heads of cows, the II group (second lactation) - 16 heads of cows, the III group (third lactation) - 16 heads of cows, the IV group (fourth lactation) - 12 heads of cows.

Milking cows runs 2 times a day by means of milking equipment "Delaval".

The animals are kept unconstrained in a typical building. Parameters of the microclimate in the room in the study period were the following: air temperature - $16.0 \pm 0.7^\circ\text{C}$, relative humidity - $56.8 \pm 2.0\%$, carbon dioxide - $0.19 \pm 0.09\%$, hydrogen sulfide - $7.0 \pm 0.7 \text{ mg/m}^3$, ammonia - $15.0 \pm 0.7 \text{ mg/m}^3$, air speed - $1.6 \pm 0.04 \text{ m/s}$, bacterial contamination - 60.2 ± 2.1 thousands of CFU/m³ (colonies forming units).

All experimental procedures were carried out in accordance with the "Regulations for the Use of Animals in Biomedical Research" and in accordance with the recommendations of the European Convention for the Protection of Animals used for experimental purposes (Porter, 1992).

Somatic cell count

To determine healthy and infected udder quarters, was used the Rapid Mastitis Test (Kerbl Shooft, Germany), and test for the SCC (somatic cell count) in milk. After the state of udder quarters was determined, secretion from positively reacting quarters was collected into sterile cups, observing the rules of asepsis. Milk was smeared in the laboratory on Standard Methods for the (Marshall, 1992) and other references. The total somatic cell count was calculated by Prescott and Breed method

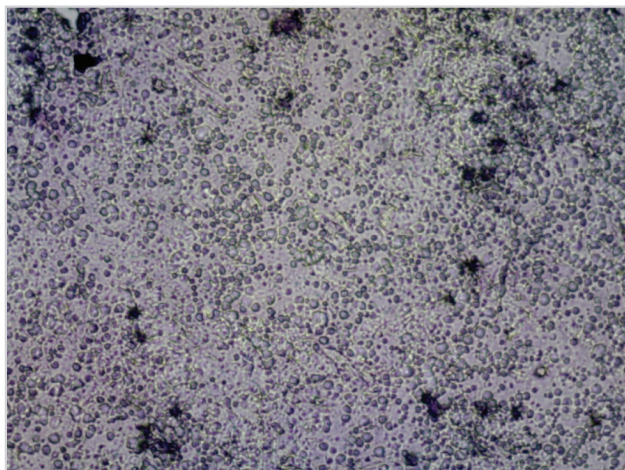


Figure 1. Somatic cells in cow's secretion with subclinical mastitis (magnification: x100)

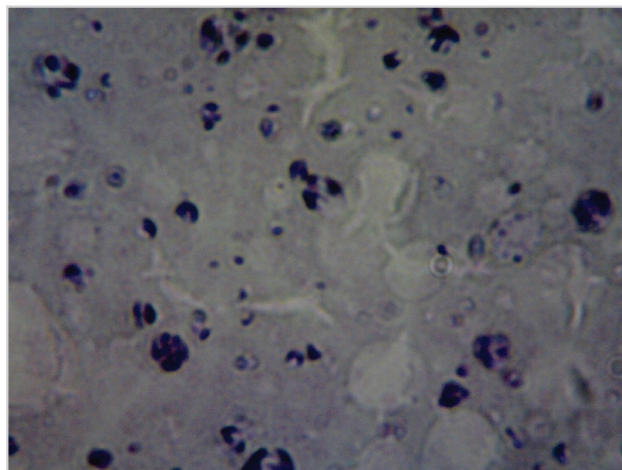


Figure 2. Somatic cells in cow's secretion with subclinical mastitis (magnification: x400)

(Prescott and Breed, 2010) and their species composition was determined.

Milk samples were taken during the morning milking from every quarter of the udder in quantity 50 mL.

To determine the number of somatic cells in cm^3 , we made a smear of 1 cm^2 in a volume of 0.02 mL. After drying, the smear in the air was fixed with alcohol-denaturate for 30 min. Then again dried and stained for Levowitz-Weber (L-W). Number of somatic cells was determined using a microscope "XS 2610 (MICROMed, Poltava, Ukraine)". To convert the number of somatic cells into 1 cm^3 of milk, we used a constant of 120.405, which was determined by us earlier (Andrievskiy et al., 2013; Shkromada et al., 2019).

Microbiological studies

Before the start of milking, disinfection of milking equipment was carried out. The study of bacterial contamination of milk cups was carried out every time when cows were milked.

To determine the microflora composition of milk, skin, udder, teats and milking equipment microbiological methods were used (Arulraj et al., 2015). For microbiological research, R-BIO-PHARM TEST SYSTEMS (Germany) were used, namely RIDA[®] COUNT, RIDA CHECK. LumitesterPD-20; LuciPacPen, RIDASCREEN Verotoxin, RIDASCREEN SET A, B, C, D, RIDASCREEN Salmonella (AFNOR EN/ISO 16140), RIDASCREEN Listeria, Sure-FoodBAC, which enable rapid and qualitative determine not only the presence of microorganisms, but also their number. To determine the conditional pathogenic microflora on the milk cups, the rapid control of the surface and liquid purity using the RIDA[®]ATP set was used, for the rapid control of pathogenic microorganisms RIDA[®]COUNT cards were used.

Used the next time and the incubation mode: to determine the total microbial number – 35°C – 24 h, to identify coliforms – 35°C – 24 h, *Escherichia coli* – 35°C – 24 h, *Salmonella* – 35°C – 24

h, *Staphylococci* – 35°C – 24 h. For microbiological monitoring the computer program "WHONET" was used.

Statistical analysis

The obtained data are statistically processed using the Fisher-Student method, taking into account the arithmetic meanings and their statistical errors, as well as the determination of the probable difference of the indicators that were compared. Significance was declared at $p < 0.05$, $p < 0.01$ and differences between means with $0.05 < p < 0.10$ were accepted as representing tendencies (Mankiewicz, 2004).

Results and Discussion

The microscopic studies of milk smears have determined specific features and number of somatic cells (Figure 1, 2). They are differentiated as lymphocytes, monocytes, neutrophils (Wall et al., 2018).

The studies carried out on the smears of cow's milk from healthy and affected quarters indicate that the somatic cell count in cow's milk is in the range from 50 to 100 ths/cm^3 .

According to the results presented in Table 1, it can be noted that in the secretion of the affected quarter of the udder, the somatic cell count increases by a thousand times. So the average amount has increased about 3 thousand times.

Determination of the species composition of somatic cells was carried out in the same smears using the immersion lens x100.

Determination of the species composition of somatic cells shows (Table 2), that both, in the milk of a healthy quarters and in the affected, species composition remains the same, but the ratio changes. So, the number of epithelial cells and lymphocytes in the milk of the affected particle decreased by 4.4 and 6.2 times, respectively. However, the number of neutrophils increased by 5.25 times.

Table 1. The content of somatic cells in cow's milk from healthy quarters and affected by subclinical mastitis ($M \pm S.E.$, SCC - ths/cm³)

Group of animals (lactation)	Healthy particle	Affected particle
I (n=10)	76.1±1.9	29012.4±275.0*
II (n=16)	77.8±1.4	33396.2±265.3*
III (n=16)	78.1±1.2	27567.1±248.1*
IV (n=12)	80.4±1.3	24832.8±279.0*
Average	78.1±1.3	228702.1±286.5*

* $p < 0.001$; SCC: somatic cell count (compared to healthy samples)

Table 2. Species composition of somatic cells in the milk of healthy and affected quarters ($M \pm S.E.$, n=16, %)

Lactation	Somatic cells	Quarter	
		Healthy	Affected
		Somatic cell count	
II	Epithelial	44.8±1.7	10.1±1.0*
	Lymphocytes	38.6±1.4	6.2±0.7*
	Neutrophils	16.6±1.4	84.9±5.8*

* $p < 0.001$; SCC: somatic cell count (compared to healthy samples)

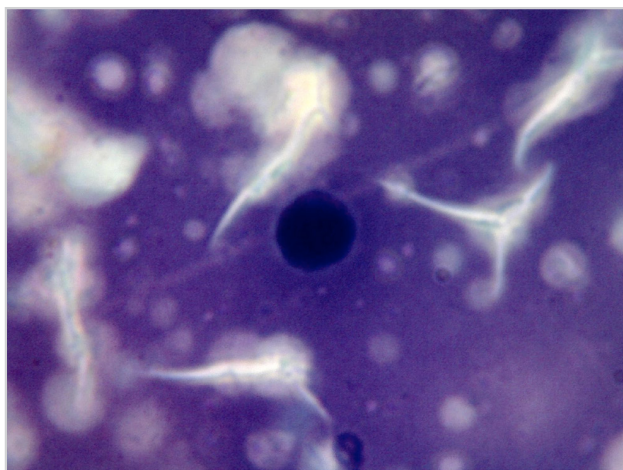


Figure 3. Lymphocyte in cow's secretion with subclinical mastitis (magnification: x1000)

Studies on determining the number of somatic cells showed that in the main period of lactation of clinically healthy animal, the SCC is up to 100 ths/cm³. In the case of subclinical mastitis, SCC increases in tens and even thousands times.

Thus, in Figure 3, the milk lymphocyte is shown in cow's secretion with subclinical mastitis. Typically, it is rounded when colored by Levowitz-Weber (LW), its nucleus of a dense consistency is intensively stained in a dark purple color; a small circle of bluish cytoplasm is clearly visible around the nucleus.

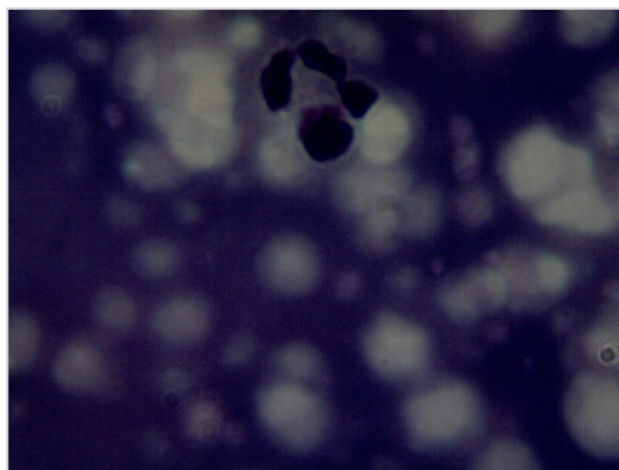


Figure 4. Segmented neutrophil in cow's secretion with subclinical mastitis (magnification: x1000)

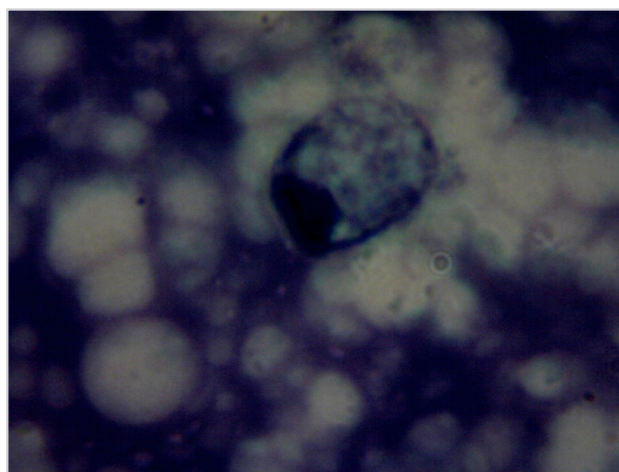


Figure 5. Basophil in cow's secretion with subclinical mastitis (magnification: x1000)

In Figure 4 segmented neutrophil is shown. Our studies have shown that neutrophils can be found in both milk of clinically healthy cows and in milk of cows with subclinical mastitis. However, it should be noted that in the case of subclinical mastitis their number increases in thousands times. In the case of disease, the number of neutrophils can amount up to 90% of all cells. Along with segmental neutrophils, stab and immature neutrophils appear in milk.

In the udder secretion of cows with subclinical mastitis, monocytes appear (Figure 5). Macrophages accumulate in large quantities in the areas of inflammation. They have a strong capacity for phagocytosis.

Basophils are granulocytes that are clearly visible on the Figure 6. They have an incorrectly rounded shape, with the nucleus of a dense consistency pushed to the periphery.

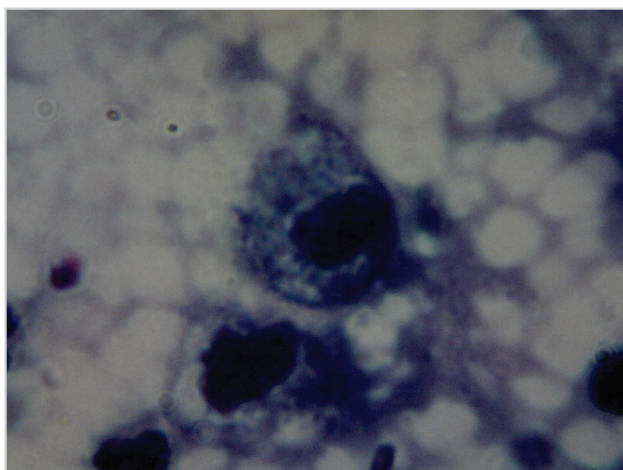


Figure 6. Monocyte in cow's secretion with subclinical mastitis (magnification: x1000)

According to the results of the study (Table 3), it can be noted that the total bacterial contamination of the milk cups was within the limits 2.1 ± 0.1 - 2.3 ± 0.3 CFU/cm² in the beginning of milking. The total bacterial contamination of the milk cups after milking five cows of I group increased by 254.6 times and after ten cows – by 636.5 times.

The same tendency was observed in relation to the general bacterial contamination of the milk cups, which were exposed to the skin of the cow's teats from other experimental groups.

Studies have shown, that on the udder skin of cows I group (Table 4) *S. aureus* forms 29%, *S. agalactiae* – 60% and associated microflora – 11% of the total number of colony-forming units.

However, the percentage of pathogenic microorganisms varied depending on the age of the animals. So, on the udder skin of cows IV group rate of *S. aureus* increased to 48% and rate of *S. agalactiae* decreased to 43% of the total number of colony-forming units.

Thus, it can be stated that even after careful cleaning and disinfection of milk cups, microorganisms still remain on it and the general microbial contamination is dynamic in the direction of increase. So, it can be assumed that the pathogens of the subclinical mastitis from the skin of the affected cow through the milk cups affect the tissues of healthy animals, thus causing transfer infection from animal to animal.

The results of the study of microbial contamination of teat and udder skin show that it always contains microorganisms that can cause subclinical mastitis (Busato et al., 2000). There is only difference in the ratio of pathogens.

Cows of 1st lactation have the smallest number of *S. aureus* and at the same time, the largest number of *S. agalactiae*. However, this ratio changes somewhat with animal aging. So, the amount of *S. aureus* slightly increases and the amount of *S. agalactiae* conversely decreases. Moreover, we have not detected any changes in the amount of associated microflora. One might assume that microorganisms on the udder skin are antagonists among themselves, especially *S. aureus* and *S. agalactiae* (Joshi and Gokhale, 2006; Schwarz et al., 2011).

Thus, pathogens of subclinical mastitis are always present on the udder skin of animals. Therefore, it is mostly impossible to treat the herd of cows completely of subclinical mastitis. But it possible to control it and keep the rate of animals infected by mastitis pathogens within 5-6%.

The main stages of disease prevention are strict compliance of the milking technology, systematic examination of cows by "cow side" tests, such as the California Mastitis Test, and measuring the electrical conductivity of milk. The separation of infected animals from healthy ones can also be used in order

Table 3. The dynamics of bacterial contamination of milk cups (M±S.E.)

Group of animals (samples)	Before milking CFU/cm ²	The fifth cow thousand CFU/cm ²	The tenth cow thousand CFU/cm ²
I (n=60)	2.1±0.1	534.7±29.9*	1336.7±45.1*
II (n=60)	2.3±0.2	601.4±23.0*	1486.3±34.6*
III (n=60)	2.3±0.3	843.6±45.0*	1568.4±67.6*
IV (n=60)	2.1±0.2	811.3±48.1*	1489.9±59.0*

*p<0.05 compared to the start of milking; CFU: colony-forming units

Table 4. Microbial contamination of the udder skin of cows (M±S.E.)

Group of animals (lactation)	Skin microflora of teats (%)		
	<i>S. aureus</i>	<i>S. agalactiae</i>	Associated
I (n=10)	29±1.4	60±3.7	11±2.0
II (n=16)	38±2.0	56±2.3	6±1.4
III (n=16)	49±3.8	44±2.9	7±1.3
IV (n=12)	48±3.7	43±4.2	9±1.2

It is known that subclinical mastitis is an infectious disease; therefore, the disease of animals can be transmitted from one animal to another. Since the transmission path is pin and the greatest contact occurs through the milk cups.

In accordance with the research objectives, we have studied the dynamics of bacterial contamination of milk cups. Before the start of milking, milk cups was thoroughly mechanically cleaned, washed with water, and disinfected. After the disinfection, milk cups was thoroughly washed with distilled water, and dried. The study of bacterial contamination of milk cups was carried out at the beginning of milking (before connecting to cows) and then every five cows. The results of the study are presented in Table 3.

to break the epizootic chain. One of the reasons for the rapid spread of subclinical mastitis in the herd is the transfer of pathogens during milking, especially from the diseased animal with increased pathogenicity to the healthy one. The main mechanical carrier of pathogens is the milk cup, since it directly contacts with the udder skin both of affected and healthy.

Therefore, if a cow affected by subclinical mastitis is present on the first stage of milking, there is a high probability that the mastitis pathogens will be transmitted to the udder skin of other animals.

Conclusion

1. In the case of subclinical mastitis the number of somatic cells in the secretion of the affected quarter of udder increases and its species composition changes.

2. Subclinical mastitis pathogens are always present on the skin of a cow's udder, but only their ratio changes with aging of the animal.

3. The main carrier of pathogens of subclinical mastitis from the infected cow to healthy one is the milk cup.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Sumy National Agrarian University with approval number 2017/01.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.Sk., O.Sh.; Design – O.Sk., A.P.; Supervision – O.Sh., G.I.; Resources – O.Sk., O.Sh., A.P., G.I.; Materials – O.Sk., G.I.; Data Collection and/or Processing – O.Sk., O.Sh.; Analysis and/or Interpretation – O.Sh., A.P.; Literature Search – O.Sh., G.I.; Writing Manuscript – O.Sk., A.P.; Critical Review – O.Sh.

Conflict of Interest: The authors have no conflict of interest to declare.

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Determination of Critical Control Points and Potential Hazard Analysis in the Production of Frozen Silverfish (*Atherina boyeri* Risso, 1810)

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Abstract

The Bandırma district of Balıkesir province has an important place in terms of production and export of aquatic and frozen fish products. Silverfish (*Atherina boyeri* Risso, 1810) has high protein quality (protein content >70%) and low price. It constitutes an important alternative source of raw material for economic fish meal production. Fresh silverfish exported to the European countries in recent years is demanded in two different product forms: Frozen and breaded frozen. In this study, the production of frozen silverfish was carried out in a business that produced aquaculture products for a considerable level of export in Bandırma. The production flow diag-

ram, in accordance with TS EN ISO 22000 Food Safety Management System and British Retail Consortium Standard, was defined to obtain a safe product in accordance with customer expectations and needs. Hazard analysis was carried out by analyzing each step using decision tree. In this way, potential hazards and the precautions to be taken to prevent them, critical control points, and critical limits belonging to these points have been set forth.

Keywords: Critical control point, frozen silverfish *Atherina boyeri* Risso 1810, hazard analysis

Introduction

Today's society wants the foodstuff to be hygienic and economical, as well as to contain protein, fat, carbohydrates, vitamins, and minerals in a balanced ratio. Fish meat is an excellent food with high protein quality, mineral and vitamin richness, low amount of energy, and abundant polyunsaturated fatty acids. Because the energy value is low, it also has a dietetic characteristic. The widely used form of fishery products for human consumption is the frozen product form, which is used in many countries as well as in various products such as salting, smoking, marinating, especially fresh consumption (Turan et al., 2006; Varlık et al., 2004).

Silverfish (*Atherina Boyeri* Risso, 1810, *A. boyeri*) is a member of *Atherinidae* family that has good adaptation talent and shows regional differentiation for morphological and biological char-

acteristics (Çetinkaya et al., 2010). Silverfish is found in rivers, lakes, ponds, and reservoirs (Küçük et al., 2006). In Turkey this fish species lives in İznik, Sapanca and Köyceğiz lakes in a dense population. This fish, which did not have economic importance in our country until recently, has gained value in recent years; and it has been in demand for consumption in domestic and foreign markets (Çolakoğlu et al., 2006).

The high quality of silverfish (protein content >70%) as well as its low price show that it is an important source of alternative raw materials in terms of economic fish feed production (Gümüş et al., 2009). Fresh silverfish, which was exported to the foreign market, has been demanded by the European Union countries in two different product forms: frozen and frozen breaded (Çolakoğlu et al., 2006).

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The Bandırma district of Balıkesir province, located at the level of TR 22, has an important place in terms of the production and export of aquaculture. Frozen seafood such as frozen fish, lobster, shrimp, mussels, crabs, and frog and land snail products are exported to Europe and the Far East (Anonymous, 2000).

The increase in the level of welfare of the countries and the awareness of the consumers has forced the firms in the food sector to seek for new pursuits in terms of food safety (Başaran, 2016). It is defined as taking necessary measures to ensure reliable food production during food safety, raw material supply, production, processing, storage, transportation, distribution, and presentation of food. The starting point of food safety is farm, and the end point is consumer. Therefore, food safety includes the procurement of healthy raw materials from "farm to consumer", the production, processing, storage, transportation, distribution, and presentation of food (Giray and Soysal, 2007).

Hazard Analysis and Critical Control Points (HACCP) is frequently used as the best system, which helps food producers to produce safe foods for consumption (Ayhan, 2013). This system aims to determine the potential hazards that may occur at any stage in the business, not only the end-product, but also the whole business where the product is produced, to control the necessary preventive and corrective actions for all possible hazards in a systematic way, and to minimize the potential physical, chemical, and microbiological diseases (Moterjemi and Mortimore, 2005; Turantaş and Ünlütürk, 1998). ISO 22000 Food Safety Management System, which was developed in recent years as based on HACCP, is a system that was developed to obtain safe food worldwide (DPT, 2007).

In this study, frozen silverfish was produced in a business that produced aquaculture products for export in Bandırma (TR221), which is located at TR 22 level in the scope of TS EN ISO 22000 Food Safety Management System, obtain a safe product according to customer expectations and needs. The production flow chart has been defined, and hazard analysis has been performed by examining with decision tree in each step. In this way, critical control points and critical limits of these points are put forward with the measures to be taken for the prevention of potential hazards.

Materials and Methods

Materials

Silverfishes that were caught by trammel net from İznik Lake and reached the laboratory within 48 h were used as research material. A total of 100 fishes were used in the analysis.

Methods

All the analysis in the obtained samples were performed by reference methods reported by the Turkish Republic Ministry of

Agriculture and Forestry (T.C. Tarım ve Orman Bakanlığı, 2012). The physical analyses were piece size of a fish, the amount of pieces in 1 kg, the min-max weight, the amount of foreign matter. The chemical analyses were histamine, mercury (Hg), cadmium (Cd), lead (Pb), benzo(a)pyrene, total dioxins, and total dioxins and dioxin-like PCBs. The microbiological analyses were numbers of total aerobic bacteria (NTAB), coliform bacteria (CB), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella* spp., *Listeria monocytogenes* (*L. monocytogenes*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), and *Vibrio cholerae* (*V. cholerae*).

Results and Discussion

According to the ISO 22000 Food Safety Management System, a complete description of the product, including the relevant safety information, must be made. The product and its composition; physical, chemical, and biological properties (pH, water activity, etc.), and processes applied to the product such as heat treatment, freezing, smoking, salting, must be fully defined. The packaging properties, distribution, and storage conditions as well as the storage life and instructions for use should be specified.

The information, such as whether each product obtained in the business is to be directly used for consumption or used as an intermediate or food additive in another business, the form of packaging (large packaging such as bulk, sack or barrel, final consumer packaging), whether it needs to be exposed to heat treatment for the last time before consumption, should be indicated in detail (Batu and Gök, 2006).

The product description of frozen silverfish is shown in Table 1. In creating product properties, the reported criteria in Turkish Republic Ministry of Agriculture and Forestry General Directorate of Food and Control Regulations on Fisheries (Anonymous, 1995), were taken into consideration.

The flow chart is defined as the schematic representation of the relationship between order and steps or processes applied in the production of a particular nutrient (Anonymous, 2003). A production flow chart should be created before the hazard analysis is performed. In the flow chart, all the steps taken by the food until it reaches the consumer's table should be shown in detail. Starting from the procurement of raw materials, entry points in the processing line of all additives and auxiliaries, all applications in the process line, waiting times and temperatures if any, packaging, heat treatment, storage, distribution operations and again, if any, quality control stages should be shown in detail (Batu and Gök, 2006). In our study, the production flow chart of frozen silverfish was formed as follows (Figure 1).

Pre-requisite programs (PRPs) are the basic conditions and activities to ensure a proper production by providing the necessary hygienic environment along the food chain, to ensure the safe preparation of the end-product, and to provide safe

food for human consumption. PRPs depend on the food chain parts of the organization and the type of organization. For example, good agricultural practices (GAP), good veterinary practices (GVP), good manufacturing practices (GMP), good hygiene practices (GHP), good laboratory practices (GLP), good distribution practices (GDP), and good trading practices (GTP).

The operational pre-requisite program (OpPRP) is defined as a pre-requisite program defined by hazard analyses where it is compulsory to control possible food safety hazards and/or contamination or proliferation of food safety hazards in the product or process environment (Anonymous, 2006).

The silverfish used as research material were hunted from Iznik Lake after the chemical and microbiological analysis results of the samples obtained from the controls carried out by the Provincial/District Directorate of Agriculture at the beginning of the hunting season were reported to be in compliance with the legal limits and in accordance with the prohibition periods determined by Republic of Turkey Ministry of Agriculture and Forestry the General Directorate of Food and Control (Table 2). So, the step of "Raw material supply and acceptance", which is the first step of frozen silverfish production flow chart, has been determined as OpPRP because of the risk of possible biological and chemical pollution risk analysis score was more than four points (Table 3).

The HACCP application is performed within an HACCP plan. The HACCP plan has been developed to ensure control of potential hazards in the food chain and is import-

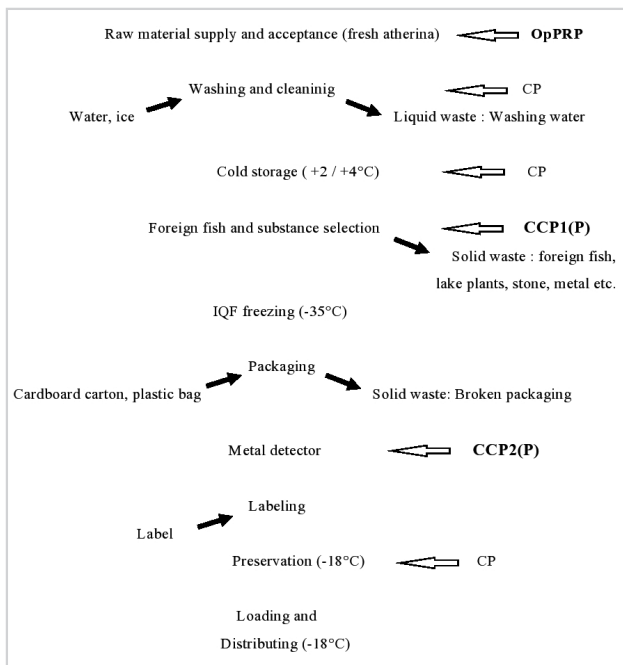


Figure 1. The production flow chart of frozen silverfish

Table 1. Product specification of frozen silverfish

Name of product: Frozen silverfish (<i>Atherina boyeri</i> Risso, 1810)	
Product specification:	
Physical properties	
Piece size of a fish	5-7 cm
Amount of pieces in a kilogram	500-550
Min-max weight	1.8-2 g
Foreign matter ratio	Max 2%
Chemical properties	
Histamine	n=9, c=2, m=100 mg/kg, M=200 mg/kg
Hg	0.5 ppm
Cd	0.05 ppm
Pb	0.3 ppm
Benzo(a)pyrene	2.0 ppb
Total dioxins (max)	4.0 pg/g
Total dioxins and dioxin-like PCBs (max)	8.0 pg/g
Microbiological properties	
Parameters Maximum tolerance	
NTAB 30°C (/g)	n=5, c=2, m=10 ⁶ , M=10 ⁷
CB (/g)	n=5, c=2, m=160, M=210
<i>E. coli</i> (/g)	n=5, c=2, m=9, M=12
<i>S. aureus</i> (/g)	n=5, c=2, m=10 ³ , M=5 × 10 ³
<i>Salmonella</i> spp.	none / 25 g
<i>L. monocytogenes</i>	n=5, c=0
<i>V. parahaemolyticus</i>	none
<i>V. cholerae</i>	none
Usage and purpose:	Consume by fried.
User / consumer group:	Except allergic to babies and fish, Suitable for people of all ages.
Allergen presence:	Allergen
GMO presence:	The product and the auxiliary materials used do not contain GMO (genetically modified organism).
Packaging:	In 1 kg nylon (PE) bag or bag in parcel, 10 kg / parcel is packed in bulk.
Shelf life and storage conditions:	24 months at -18°C
Place of sale:	Domestic market, hotel, restaurant, foreign market customer groups.
Warnings in the label:	Allergen. After thawing, do not freeze again.
Special distribution control:	Transport / storage temperature -18°C min.
Hg: mercury; Cd: cadmium; Pb: lead; PCBs: polychlorinated biphenyls; NTAB: number of total aerobic bacteria; CB: coliform bacteria	

Table 2. OpPRP plan of frozen silverfish production

No.	Hazard	Control measures	What	Tracing how	Frequency	Corrective action	Responsible	Tracing records
1. Supply and admission of raw material	Pathogen bacteria because of pollution in the lake, caught silverfish	In compliance with hunting periods designated by	Microbiological analysis	Related methods	One time at the season opening	Not hunting from unsuitable lakes	Business Lab. Reference Lab. General	Analysis by The Ministry Origin documents Controls made in end-product
	<i>E. coli</i>	General Directorate of Protection and Control					Directorate of Preventing and Control	Formal analysis results
	<i>Salmonella</i> spp.							
	<i>V. cholerae</i>							
	<i>V. parahaemolyticus</i>							
	<i>L. monocytogenes</i>							
	Containing chemical pollution	Hunting from lakes, where in obtained						
	Hg	appropriate results						
	Cd	of analysis of the						
	Pb	samples by the						
	Benzo(a)pyrene	Provincial and District Directorate						

Hg: mercury; Cd: cadmium; Pb: lead; PCBs: polychlorinated biphenyls

ant in terms of food safety and prepared in accordance with HACCP principles (Burson, 2002). The first principle required to implement the HACCP system is to carry out hazard analysis. According to this system, physical, chemical, and biological agents constitute potential hazards to health in foods. Hazard analysis involves the assessment of potential hazards that may occur at any stage of production, and their assessment of the likelihood of occurrence and the seriousness of the hazard they create. Hazard analysis critical control points must ensure physical, chemical, and microbiological safety. Failure to perform this step, which requires technical expertise and experience, may cause the food produced in the future not being at the desired level of safety. Hazard analyses include the identification and evaluation of hazards arising from raw materials, additives, processing, distribution, retail sale, and consumption (Barrie, 1996; Göktan and Tunçel, 1992; Kayaardı, 2004).

The brainstorming technique is used to identify potential hazards as the first stage when performing hazard analyses. At this stage, the HACCP team creates a list by identifying the potential hazards in all stages from the raw material to the use of the product. The "decision tree" is used to identify the hazards (Anonymous, 1997). The decision tree is a set of systematic questions that are taken into account in terms of deciding whether the point is a critical control point for a defined hazard at a point in the production process of the food. For the application of the decision tree, each process step specified in the flow chart must be included in the process, respectively. The decision tree in each step should be applied to every expected hazard and every control measure (Anonymous, 2005a). Table 3 shows the frozen silverfish hazard/risk analysis.

The Critical Control Point (CCP) is the stage where the food safety hazard is prevented or eliminated or reduced to an acceptable level (Anonymous, 2006). A critical point determination for the control of a hazard can be facilitated by the use of decision trees. In the application of the decision tree, each process step specified in the flow chart must be included in the process, respectively. At every step, the decision tree should be applied to every hazard expected to occur and every control measure determined (Anonymous, 2005a).

The HACCP plan contains all necessary information, references, and records related to the system. All substances, except PRPs for the implementation of the HACCP system, are in fact included in the HACCP plan (Anonymous, 2005b; Burson, 2002). Table 4 shows the critical control points identified by the hazard analysis and risk analysis performed on the flow chart and information on the implementation of HACCP principles at these points.

Table 3. Hazard/risk analysis of frozen silverfish production

Preliminary question (PQ): Can this hazard be controlled with PRP?

- Q1: Is there any control measure that can be applied by the operator at any stage of the production for this hazard? Can the hazard be avoided in business? Which process step?**
- Q2: Is the contamination caused by this hazard upper than the acceptable level or can it reach unacceptable levels?**
- Q3: Is this operation step designed specifically to remove this hazard or to reduce the possibility of realization to the acceptable levels (operations designed specifically such as autoclaving, pasteurization, metal detector)?**
- Q4: Can this defined hazard be removed in any following step, or can it be reduced to the acceptable levels?**

Processing	Hazard	Possibility	Severity	Risk score	Control measure	Control measure assessment and selection of combinations decision tree										
						PRP	Y (Q1)	Y	N - CP	Y - CCP	N	Y (Q3)	N - CCP	OPPRP	CCP	
1-Raw material supply and acceptance (fresh atherina)	B - Presence of <i>E. coli</i> , <i>Salmonella</i> , <i>V. cholerae</i> , <i>V. parahaemolyticus</i> and <i>L. monocytogenes</i> because of pollution in the lakes from where silverfish are caught.	2	4	8	Control of lake water and opening to hunting according to the Ministry monitoring program. Fish intake is made from the clean areas allowed by The Ministry. In addition, microbiological analysis of the raw material in the periods specified in the microbiological analysis plan is carried out.	Y									X	
	P - Presence of parasite in silverfish.	2	2	4	During each organoleptic examination, parasite control is performed. Unsuitable parties are rejected.	Y										
	C - Exceeding limits specified by the Ministry of Hg, Cd, Pb, Benzo(a) pyrene and dioxins and dioxin-like PCBs in silverfish.	1	5	5	Heavy metal analysis is made at the beginning of the season in the raw material taken from each region.	Y									X	
2-Washing and cleaning	B - Contamination of NTAB, CB, <i>E. coli</i> and <i>C. perfringens</i> from water.	1	4	4	Water was chlorinated and passed through UV filter, and drinking water quality. NTAB, CB, and <i>E. coli</i> analysis are performed, weekly. NTAB, CB, <i>E. coli</i> , and <i>C. perfringens</i> analyses are performed by official authorities every three months.	Y										
	C - Upper limits of contamination of free chlorine, Fe, nitrite, ammonium, aluminum from water	1	4	4	Water is chemically suitable. Compliance is checked by analysis every three months. Chlorine control is performed twice a day.	Y										
	P - Excessive exposure to water will cause its stomach to explode.	1	1	1	The fishes are washed with cold water without giving too much pressure to the water.	Y										
3-Cold storage (2/4°C)	B - Number of NTAB may increase if the tank temperature rises.	1	2	2	In the warehouse, the PLC system automatically measures and records temperature every two hours. It is under constant observation.	Y										

Table 3. Hazard/risk analysis of frozen silverfish production (Continued)

Preliminary question (PQ): Can this hazard be controlled with PRP?

- Q1: Is there any control measure that can be applied by the operator at any stage of the production for this hazard? Can the hazard be avoided in business? Which process step?**
Q2: Is the contamination caused by this hazard upper than the acceptable level or can it reach unacceptable levels?
Q3: Is this operation step designed specifically to remove this hazard or to reduce the possibility of realization to the acceptable levels (operations designed specifically such as autoclaving, pasteurization, metal detector)?
Q4: Can this defined hazard be removed in any following step, or can it be reduced to the acceptable levels?

Processing	Hazard	Possibility	Severity	Risk score	Control measure	Control measure assessment and selection of combinations and selection of combinations decision tree									
						PRP	Y (Q1)	N (CP)	Y (CCP)	N	Y (Q3)	N (CCP)	OPPRP	CCP	
	B - Possibility of cross-contamination in store.	1	3	3	No other raw material / product is stored in the cold storage where the silverfish is kept.	Y									
4-Foreign fish and item selection	C - CB and <i>E.coli</i> contamination from the selection band and personnel hands.	2	3	6	The staff disinfect hands and wear gloves. The selection tape is cleaned and disinfected according to the cleaning plan. Weekly swap controls are performed.	Y									
	P - The presence of algae, plant parts, stone fragments, crayfish, and other alien fish and insufficient selection and remaining of these impurities in the final product.	3	3	9	Foreign substances are taken by female workers standing in the band. Banding speed is adjusted according to person and the rate of foreign fish.	N	X	X	X				X		X
	C - Contamination of detergent and disinfectant residues from the selection band.	1	3	3	The detergents and disinfectants used will not leave any residue by good rinse. Product safety data sheets are available. The cleaning staff is careful about rinsing. After rinsing, the residue is verified by checking.	Y									
5-IQF Freezing (-35°C)	B - NTAB, CB, <i>E.coli</i> /contamination due to the inadequate cleaning of IQF.	2	3	6	The IQF is cleaned and disinfected according to the cleaning schedule after each use. It is controlled by swab.	Y									
	C - Contamination of detergent and disinfectant residue from the IQF band.	1	3	3	The detergents and disinfectants used will not leave any residue by good rinse. Product safety data sheets are available. The cleaning staff is careful about rinsing. After rinsing, the residue is verified by checking.	Y									
6-Packaging	B - NTAB and CB contamination from used nylon bags and packaging machine.	2	3	6	Nylon bags are supplied hygienically from the approved suppliers and stored in the plant as appropriate. The swap test is done from the	Y									

Table 3. Hazard/risk analysis of frozen silverfish production (Continued)

Preliminary question (PQ): Can this hazard be controlled with PRP?

Q1: Is there any control measure that can be applied by the operator at any stage of the production for this hazard? Can the hazard be avoided in business? Which process step?

Q2: Is the contamination caused by this hazard upper than the acceptable level or can it reach unacceptable levels?

Q3: Is this operation step designed specifically to remove this hazard or to reduce the possibility of realization to the acceptable levels (operations designed specifically such as autoclaving, pasteurization, metal detector)?

Q4: Can this defined hazard be removed in any following step, or can it be reduced to the acceptable levels?

Y: Yes N: No N C: Not CCP

Processing	Hazard	Possibility	Severity	Risk score	Control measure	Control measure assessment and selection of combinations and selection of combinations decision tree												
						PRP	Y	N (Q1)	Y	N - CP	Y - CCP	N	Y (Q3)	N - CCP	OPPRP	CCP		
					incoming party. The packaging machine is cleaned and disinfected according to the cleaning plan. Weekly swap controls are performed.													
	C - Nylon bags used are not appropriate in chemical parameters and migration.	3	4	12	The suitability of the chemical parameters has been confirmed in the specification given to the supplier.	Y												
	C - Contamination of detergent and disinfectant residue from the packaging machine.	1	3	3	Detergents and disinfectants used are given good rinse to leave no residue. Product safety data sheets are available. The cleaning staff is careful about rinsing. After the rinsing operation, the residue is verified by checking.	Y												
7-Metal detector	P - Metal parts remain between products.	5	5	25	Metal detector calibration is confirmed before and during each use, the product boxes are passed one by one through the metal detector. The metal detector is used only by trained personnel. As it is the equipment that affects food safety, it is serviced in three months.	N	X	X	X	X								X
8-Labeling	-	-	-	-	-													
9-Preservation (-18°C)	B - Increased bacterial load as a result of temperature rise above -18°C.	1	2	2	Notification: 2004/46 Temperature measurement and recording is done automatically with PLC system in warehouses for two hours. When there is a malfunction in the warehouse, the products are taken to another warehouse.	Y												

Table 3. Hazard/risk analysis of frozen silverfish production (Continued)

Preliminary question (PQ): Can this hazard be controlled with PRP?

Q1: Is there any control measure that can be applied by the operator at any stage of the production for this hazard? Can the hazard be avoided in business? Which process step?

Q2: Is the contamination caused by this hazard upper than the acceptable level or can it reach unacceptable levels?

Q3: Is this operation step designed specifically to remove this hazard or to reduce the possibility of realization to the acceptable levels (operations designed specifically such as autoclaving, pasteurization, metal detector)?

Q4: Can this defined hazard be removed in any following step, or can it be reduced to the acceptable levels?

Processing	Hazard	Possibility	Severity	Risk score	Control measure	Control measure assessment and selection of combinations decision tree								
						Y (Q1)	N - CP	Y - CCP	N	Y (Q3)	N - CCP	OPPRP	CCP	
10-Loading and Distributing (-18°C)	B - Cold chain breakage during loading.	1	2	2	Before loading, the vehicle interior temperature is set to -18°C and the pallets are loaded without breaking the cold chain.	Y								
	B - Cross contamination due to non-disinfection of the vehicle.	1	2	2	The vehicle is disinfected and controlled according to the loading instructions.	Y								
	P - Misidentification of the palette.	1	1	1	The production responsible identifies the pallets at the beginning of the installation.	Y								
	P - Lost crushing of cartons, breakage of products, damage due to improper placement of pallets.	1	1	1	The production manager stands at the loading, the pallets, and parcels are visually checked and the experienced storekeeper supports the gaps by placing the pallets.	Y								

NTAB: number of total aerobic bacteria; IQF: individual quick freezing; PCB: polychlorinated biphenyl; PLC: programmable logic controller; CCP: critical control point

Table 4. HACCP plan of frozen silverfish production

		Tracing								
CCP	Important hazard	Control measure	Critical limit	What	How	Frequency	Who	Records	Correction	Verification
	The presence of algae, plant parts, stone fragments, crayfish, and other alien fish, insufficient selection and extraction of these impurities in the final product.	Foreign substances standing on the tape are taken by female workers. Banning speed is set according to the rate of person and foreign fish.	Stone should never be No metal Barbed fish should never be The branch should never be The limit of other harmless foreign fish should be max 1%/	Fishes	Hand selecting	All fish passing through the band	Featured staff Control officer	Final product control records Personnel identification form	Personnel increment	No customer complaint
CCP1 / P - Physical selection	Metal parts remain between products.	Metal detector calibration is confirmed before and during each use, the product boxes are passed through the metal detector, separately. The metal detector is used only by trained personnel.	When the test kits of the machine are switched on, the lamp illuminates and the acoustic signal indicates that it is working and at the proper calibration. Test kits are Fe <1 mm, Non-Fe <2.5 mm, AISI <3 mm and metal banded blue bandage.	Checking the calibration of the machine.	The detector light upon with test kits and gives an audible signal.	Before each use	Responsible for use of metal detectors	Metal detector usage registration form	If the machine does not signal with the test kits, the maintenance officer, the quality assurance unit, and the production engineer are informed. From the last check, the labeled products are separated and marked. The machine is repaired and adjusted by the technical service. Test kits are validated. After the machine is set, the separated products are passed through the metal detector again.	Metal detector usage records are checked daily by production / quality assurance. Each customer complaint is reviewed by the Food Safety Management Representative. Inappropriate product reports are checked by the Food Safety Management Representative.
CCP2 / P - Physical metal detector										

Table 4. HACCP plan of frozen silverfish production (Continued)

CCP	Important hazard	Control measure	Tracing					Correction	Verification
			Critical limit	What	How	Frequency	Who		
CCP2 / P – Physical metal detector								Packages, found metal pieces are opened, metal parts are found, the source is investigated by the production. Engineer and quality assurance unit. Necessary corrective or preventive action is initiated.	

HACCP: hazard analysis and critical control point; CCP: critical control point

In our study, as a result of the hazard analysis and risk analysis carried out on the frozen silverfish production flowchart, step 4 “foreign fish and substance selection” was determined as the Critical Control Point 1, Physical (CCP 1P), (risk analysis score was 9 points, Table 3) by physical hazard due to the foreign substances, the presence of algae, plant parts, piece of stone, crayfish and other alien fish, and remaining these foreign substances in the end-product by inadequate selection. “Metal detector”, which is the seventh step of the frozen silverfish production flowchart, has also been determined as Critical Control Point 2 P (CCP 2 P) (risk analysis score was 25 points, Table 3) due to the physical hazard of metal part remaining between products according to the risk analysis and decision tree application. The HACCP plan of frozen silverfish production is shown in Table 4.

Conclusion

Frozen food industry is a food industry branch operating in various stages of freezing, preservation of freezing, storage, transportation, distribution and consumption of high-quality vegetables, fruits, fish products, meat products after pre-treatment such as selection, sorting, washing, cutting, chopping, and scalding (DPT, 2001).

A quality raw material is required to produce a high-quality product (Varlık et al., 2004). Fish and other seafood products contain many microorganisms from marine environment. These products can be contaminated during transport and processing (Turantaş ve Ünlütürk, 1998). Many elements found in aquaculture can be essential for human life in trace amounts. However, the accumulation of elements such as lead, cadmium, and mercury in the organism is known to be harmful to human health (Çaklı, 2007).

In our country, with the harmonization laws of the European Union, the existing laws, regulations, and related instructions and notifications have entered into a rapid change and the adaptations on various subjects continue. This situation is important to make our products in the global market more qualified and reliable in Europe and worldwide, especially in terms of competition abroad (Yeşilsu and Özyurt, 2013).

The HACCP system is often used as the best system for plan design to assist food manufacturers in producing safe foods for consumption (Ayhan, 2013). As based on HACCP, ISO 22000 Food Safety Management System developed in recent years is a system developed to obtain safe food worldwide. In the light of this information, it is considered that this study is a basic study that can be applied to businesses that produce frozen silverfish and export abroad.

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Processing - U.G., H.E., R.M.A.; Analysis and/or Interpretation - U.G., H.E., R.M.A.; Literature Search - U.G., H.E., R.M.A.; Writing Manuscript - U.G., H.E., R.M.A.; Critical Review - U.G., H.E., R.M.A.

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Morphological Characteristics of Pacing Horses and Examination of Breeding Conditions*

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Abstract

The purpose of this study was to examine the morphological characteristics and breeding conditions of pacing horses in the Afyonkarahisar province. A total of 117 head of pacing horses, as well as farm operations and opinions of horse owners, were evaluated. The overall means of height at wither, body length, rump length, chest depth, chest circumference, head length, and forehead width were measured: 142.42, 145.15, 49.77, 55.43, 161.44, 51.94, and 21.52 cm respectively. It was determined that horses with Turkish native genotypes and 1-3 elder horses had the lowest body measurements. It was determined that the pacing horses had the bay, chestnut, gray, black, and chestnut paint coat colors. It has been determined that, in the choosing of pacing horses, horse owners pay great

attention to the parent information (71.1%), the temperament (71.1%), body condition (68.9%), and the foot-nail structure (62.2%) of horses. As a result, it was concluded that the pacing horses with native genotypes in the Afyonkarahisar province were smaller than those who were crossbred and of foreign origin. Also, it was determined that the horses examined were of the bay, chestnut, gray, and black coat colors. In addition, it was concluded that the breeding conditions of pacing horses should be improved, and the horse owners should be informed about horse training and exercising.

Keywords: Body measurements, breeding, coat colors and marking, pacing horse

Introduction

Horses have been used in the past as labor force and are currently being breeding for various competitions, mostly sports, and they are still used in agriculture in operations in the highlands in some countries (Özbeyaz and Akçapınar, 2005). The number of horses in the world decreased after the Second World War, and according to the FAO (Food and Agriculture Organization of the United Nations) data from 2017, there are a total of 60 566 601 heads in the world. The number of horses in Turkey has decreased similarly to the rest of the world, and there were 120 040 heads in 2017 (FAO, 2019). In Turkey, a significant portion of the horse presence constitutes Thoroughbred, Arabian horses, and

native horses. Thoroughbred and Arabian horses are used in racing, while native horses are used in traditional horse sports such as pacing and javelin.

The pacing is characterized by the limbs moving together on the same side, and when two feet on one side rise at the same time, the two feet on the other side are on the ground (Arpacık, 1999). For 2, 3, 4, and 5-year-old foals, pacing runs in Turkey are performed on a 10-meter-wide track (Anonymous, 2017d). In a study conducted on pacing horses, Andersson et al. (2012) named the DMRT3-Ser 301 STOP mutation as having an essential relationship with pacing. Özbeyaz et al. (2016) found the DMRT3 mutant allele frequency in the pacing hors-

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es in Turkey at 90.7%, 98.40%, 95.80%, and 96.40%, in native, Iranian, Afghanistan, and Bulgaria origins, respectively. Yüceer et al. (2016a) reported that the pacing horses in Turkey did not differ significantly from the region regarding genotypically and that they were considerably different from the Arabian horses and Thoroughbred and that the allele variety of the pacing horses was much higher than the Arabian horses and Thoroughbred. In a study carried out by Çağlayan et al. (2010) on pacing horses in Turkey, the overall means for the height at wither, height at rump, body length, chest depth, chest circumference, cannon bone circumference, and head length were found 139.21, 138.28, 141.60, 58.38, 155.30, 17.69, and 56.49 cm, respectively. In another study carried out by Yüceer et al. (2016b), on the Turkish native pacing horses, the means for the height at wither, height at rump, body length, chest depth, chest circumference, cannon bone circumference, and head length were found to be 138.92, 139.67, 145.51, 61.91, 156.45, 17.06, and 52.53 cm, respectively. In the studies carried out on Turkish native horses in the Van and Kars provinces in Turkey; the gray, bay, chestnut, black, isabelline, and buckskin coat colors were identified (Bayram et al., 2005; Kirmizibayrak et al., 2004).

This study was conducted to examine some body measurements of pacing horses, determination of coat colors and white markings, breeding conditions in operations, training, and choosing of pacing horses.

Materials and Methods

Materials

This study included 117 heads of Turkish native, crossbred, and foreign origin pacing male and female horses at different ages in the Afyonkarahisar province in 2016 and 2017, Turkey. Moreover, in this study, the breeding, feeding, and barn conditions of 41 operations and the practices of 45 horse owners regarding pacing horse training and choosing were evaluated. The measurements, detection and notifications were recorded in the form. The genotype of the horses used in the study was based on the declaration of the horse owners. Also, the age of horses was established by determining the age, as well as the declaration of the horse owners. This study was conducted according to the ethical principles with the letter dated 06/14/2016 and numbered 49533702/105 of the Local Ethics Committee of Animal Experiments at Afyon Kocatepe University.

Methods

The height at wither, height at rump, body length, back length, rump length, chest circumference, chest depth, cannon bone circumference, head length, and forehead width were determined with the horse standing on a flat surface using the measuring stick (Hauptner) and tape (Arpacik, 1999). The training of pacing horses, coat colors and white markings, housing type, feeding, grooming and frequency, farrier supply, horseshoe, saddle and bit type, training obtained from

face-to-face interviews with horse owners, training frequency and duration, the importance of choosing of pacing horses, and frequently encountered injuries were recorded in the form. In the creation of this form, Yıldırım and Yıldız (2013) notifications were used.

Statistical analysis

For the statistical analysis of the obtained body measurements, the $Y_{ijkl} = \mu + G_i + S_j + A_k + e_{ijkl}$ model was used in the variance analysis. In this model, Y_{ijk} was the observation value, μ is the overall mean value, G_i is the effect of genotype (i =native, crossbred, and foreign), S_j is the effect of gender (j = male and female), A_k is the effect of age (k =1-3, 4-6, and 7 \leq), and e_{ijkl} represents the random error. In each subgroup, the means was compared with the Duncan's Multiple tests. Information about the management, feeding, training, and choosing preferences of horse owners in operations is given in as a proportion (%). The PASW Statistics 18.0 program was used in calculations.

Results

Morphological characteristics of pacing horses

The values of body measurements of pacing horses in the province of Afyonkarahisar are presented in Table 1. In these pacing horses, the height at wither, height at rump, body length, chest depth, chest circumference, cannon bone circumference, head length, and the forehead width for overall means were detected as 142.42 \pm 0.83, 142.50 \pm 0.81, 145.15 \pm 1.06, 55.43 \pm 0.56, 161.44 \pm 1.39, 17.58 \pm 0.16, 51.94 \pm 0.33, and 21.52 \pm 0.16 cm, respectively. The effects of the genotype (native, crossbred, and foreign origin), gender (male and female), and age (1-3, 4-6, and 7 \leq years) on some body measurements were found to be statistically significant ($p < 0.05$, $p < 0.01$, $p < 0.001$). According to genotype, the lowest body size values were determined in Turkish native pacing horses. In this study, it was determined that pacing horses were of the bay (53.0%), chestnut (23.1%), gray (18.8%), black (4.2%) and chestnut paint (0.9%) coat colors. In addition, 43.6% of these horses had white facial markings, and 34.2% of these horses had white leg markings.

Breeding conditions, management, and feeding

In this study, breeding conditions, housing, management, and feeding information were examined in the operations visited. It was found that the barns were tie stall (75.6%) and box stall (24.4%) housing. A total of 3 to 5 kg/day roughage (hay, fodder, alfalfa, vetch) and 3 to 6 kg/day concentrated feed (barley, vetch, and oats ration) were reported to be given to horses in operations. Also, the proportion of giving vitamin-mineral mixtures (powder, injectable, and licking block), raisins, and carrots were found to be 73.17% in operations. It has been stated that 92.7% of the visited operations were grooming, and 68.3% of them were providing farrier from outside the operations. In addition, it was determined that the horseshoe type on pacing horses was usually closed, and an imported saddle and a port bit were used.

Table 1. Least-squares means and standard errors for body measurements in pacing horses

Genotype	n	Height at wither	Height at rump	Body length	Back length	Rump length	Chest depth	Chest circumference	Cannon bone circumference	Head length	Forehead width
		$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	$\bar{X} \pm S_x$
μ	117	142.42±0.83	142.50±0.81	145.15±1.06	55.40±0.71	49.77±0.44	55.43±0.56	161.44±1.39	17.58±0.16	51.94±0.33	21.52±0.16
Turkish Native	97	138.81±0.56 ^b	138.95±0.55 ^b	142.98±0.72 ^b	53.05±0.48 ^b	49.09±0.30	54.76±0.38	158.32±0.94	17.69±0.11	51.09±0.22 ^b	21.20±0.11 ^b
Crossbred	11	140.70±1.65 ^b	139.91±1.61 ^b	141.59±2.11 ^b	53.37±1.41 ^b	49.93±0.88	54.21±1.11	161.24±2.78	17.36±0.33	51.15±0.66 ^b	21.17±0.32 ^b
Foreign origin	9	147.75±1.78 ^a	148.63±1.74 ^a	150.87±2.29 ^a	59.77±1.53 ^a	50.28±0.95	57.34±1.20	164.77±3.00	17.69±0.35	53.57±0.71 ^a	22.20±0.35 ^a
Gender											
Male	78	142.41±0.88	142.33±0.86	143.73±1.12 ^b	54.48±0.75 ^b	49.41±0.47	54.06±0.59 ^b	158.38±1.48 ^b	17.85±0.17 ^a	52.03±0.35	21.49±0.17
Female	39	142.43±1.08	142.67±1.05	146.56±1.38 ^a	56.31±0.92 ^a	50.12±0.57	56.81±0.72 ^a	164.50±1.81 ^a	17.31±0.21 ^b	51.85±0.43	21.56±0.21
Age											
1-3	38	139.41±1.06 ^b	139.73±1.04 ^b	140.32±1.37 ^b	52.78±0.91 ^c	48.03±0.57 ^b	53.25±0.72 ^b	155.23±1.79 ^c	16.67±0.21 ^b	50.37±0.43 ^b	20.66±0.21 ^b
4-6	39	143.77±1.13 ^a	143.83±1.11 ^a	147.17±1.45 ^a	56.01±0.97 ^b	50.69±0.60 ^a	56.26±0.76 ^a	162.47±1.90 ^b	17.95±0.22 ^a	52.45±0.45 ^a	21.86±0.22 ^a
7≤	40	144.08±1.05 ^a	143.94±1.03 ^a	147.96±1.35 ^a	57.32±0.90 ^a	50.58±0.56 ^a	56.80±0.71 ^a	166.62±1.77 ^a	18.12±0.21 ^a	52.99±0.42 ^a	22.04±0.21 ^a

μ : The over all mean; $\bar{X} \pm S_x$: Least-squares mean±standard error of mean

-: Not significant (p>0.05); *: p<0.05; **: p<0.01; ***: p<0.001

a, b, c: Different superscript letters differ significantly in each subgroup in the same column (p<0.05)

During the meetings with the pacing horse owners, only 14 owners said they were interested in pacing horse training. On the other hand, they expressed the age of horse training as 18, 24, and 25 months. It was said that the horses were given training for 60-120 minutes per day, and the experience of the trainers was over 5 years. It was stated that the training of pacing foals was continued by attaching chains to the foal's foot after the training of the bridle and saddle. During the meetings with horse owners, 31 breeders stated that they exercise their horses regularly. The duration of exercising is 30-120 minutes, and the majority of the horses have 60-120 minutes of exercising. When determining the injuries, the breeders stated that they are mostly encountering, pastern, tarsus, bridle, and saddle injuries in horses. Table 2 presents the findings regarding the issues that horse owners pay attention when choosing a pacing horse. It was determined that horse owners pay more attention to the parent information (71.1%), temperament (71.1%), body condition (68.9%), and foot-nail structure (62.2%) when choosing pacing horses.

Discussion

Morphological characteristics of pacing horses

The least-squares means and standard errors for body measurements presented in Table 1 showed that the Turkish native pacing horses were generally lower than those that were crossbred and foreign origin. Regarding age, the lowest body measurement values were found in horses 1-3 years old. The height at wither, height at rump, rump length, chest circumference, and head length were 138.81, 138.95, 49.09, 158.32, and 51.09 cm for Turkish native pacing horses; 140.70, 139.91, 49.93, 161.24, and 51.15 cm for crossbred pacing horses; and 147.75, 148.63, 50.28, 164.77, and 53.57 cm for foreign origin pacing horses, respectively. Similar to the statements by Yüceer et al. (2016b), this situation shows that Turkish native pacing horses were smaller than other pacing horse genotypes. The body measurements of pacing horses in the province of Afyonkarahisar, such as the height at wither, height at rump, body length and chest circumference overall means, were higher than the values reported by Çağlayan et al. (2010) for pacing horses. In this study, the height at wither, height at rump, and body length averages for Turkish native pacing horses were higher than those reported in the studies on Turkish native horses in the Van and Kars provinces (Bayram et al., 2005; Kırmızıbayrak et al., 2004). On the other hand, the values reported by Yüceer et al. (2016b) for Turkish native pacing horses and the values reported for Arabian horses in Turkey (Gücüyener Hacı and Akçapınar, 2011) were lower. This may be due to factors such as genotype, breeding conditions, and feeding. Also, it was found that the height at wither of the pacing horses in this study was in the range 133-142 cm reported for the

Table 2. Preferences of horse owners in the pacing horse choosing

	Important		Not important		No idea	
		%		%		%
Parent information	32	71.1	-	-	13	28.9
Coat colors	9	20.0	22	48.9	14	31.1
White markings	8	17.8	23	51.1	14	31.1
Foot-nail structure	28	62.2	3	6.7	14	31.1
Temperament	32	71.1	1	2.2	12	26.7
Body condition	31	68.9	-	-	14	31.1

Paso Fino horse and higher than Icelandic horse (Anonymous, 2017a; Anonymous, 2017b). The length of the back and rump values was the lowest for the native pacing horses. The forehead width is similar to the native and crossbred genotypes and is lower than for foreign origin pacing horses.

In this study, it was found that pacing horses were of the bay (53.0%), chestnut (23.1%), gray (18.8%), black (4.2%), and chestnut paint (0.9%) coat colors. Also, in 43.6% and 34.2%, White facial and leg markings were detected, respectively. Some researchers reported that American Saddlebred, Ayvacık pony, and Canik horses were of the bay, black, gray, chestnut, and chestnut paint coat colors, and Arabian horses in Turkey were of the chestnut, gray, and bay coat colors (Anonymous, 2017; Aritürk, 1956; Gücüyener Hacan and Akçapınar, 2012; Güleç, 1995). The presence of the chestnut, bay, and gray colors in the native horses in the Kars region is mentioned, in addition to these colors in the horses in the Van region, the presence of the black, gray, buckskin, and isabelline coat colors has also been reported (Bayram et al., 2005; Kırmızıbayrak et al., 2004).

Breeding conditions, management, and feeding

It was determined that the pacing horse barns in the province of Afyonkarahisar are tie stall (75.6%) and box stall (24.4%) housing. For horses in the tie stall housing, the possibility of movement is restricted, which is considered to be a disadvantage regarding the horse performance. In addition, according to observations and determinations made during the research, housing measures and ventilation facilities concerning housing conditions are thought to be improved. While 92.7% of the operations stated that they were grooming regularly, 68.3% said they were bringing the farrier from the outside. Grooming in horse operations is an affirmative situation. The saddle used in pacing horses is usually imported (European origin), and the port bit is used. It is considered preferable because the imported saddle is robust, and the port bit gives the rider an advantage in horse control. On the other hand, almost half (41.5%) of the operations used the closed type horseshoe on four feet, and 36.5% stated that they used closed horseshoe on the front legs only or the hind legs only. The closed type horseshoe is considered to be preferred because of the protection it pro-

vides on the racecourse. It was stated that horses were given 3 to 5 kg/day roughage, 3 to 6 kg/day concentrated, and 73.17% of the operations were given vitamin-mineral mix (powder, injectable, and licking block), raisins, and carrots. It is a positive situation to use vitamin-mineral mixtures to feed horses in an essential part of operations and to pay attention to nutrition.

In the meetings held with the owners of pacing horses, it was determined that only 14 of them were interested in pacing horse training. These breeders expressed that the age of the horses to start training was 18, 24, and 25 months. Also, it was also determined that after the bridle and saddle training, the horse continued with the chain attached to the foot. This practice is thought to be performed to ensure that the limbs on the same side move together. During the meetings with the horse owners, 31 (68.9%) stated that they regularly exercise their horses, 14 (31.1%) did not exercise them, but only rode a horse intermittently. The training and the exercising schedule applied to the horses needs to be developed. In the determination of injuries in horses, 36 of the breeders did not encounter injuries, and 9 of them said that pastern-, tarsus-, bridle-, and saddle-related injuries were the most frequent. Such situations can occur during the use of horses, so this makes the bridle, saddle, and foot problems even more prominent. Horse owners stated that they paid more attention to the parent information (71.1%), temperament (71.1%), body condition (68.9 %), and the foot-nail structure (62.2%) when choosing the pacing horses.

As a result, it was found that the Turkish native pacing horses in the province of Afyonkarahisar were smaller than those that were crossbred and foreign origin and that the bay, chestnut, gray, and black coat colors were found frequently. The effects of genotype, age and gender on body measurements were found to be statistically significant. In addition, it was concluded that the breeding conditions of pacing horses should be improved and that the owners should be informed about horse training and exercising.

Ethics Committee Approval: Ethics committee approval was received for this study from the local ethics committee of animal experiments at Afyon Kocatepe University (Approval number: 49533702/105, date: 06/14/2016).

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Electrocardiographic Studies in Shall Sheep

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Abstract

The use of sheep in experimental animal models has increased recently. In the present study, we investigated normal electrocardiogram (ECG) parameters of clinically healthy Shall sheep. The animals were divided into two gender and age groups. Electrocardiograms were recorded on a base-apex lead, using limb lead I for at least 2 minutes. The heart rate range was 71–166 beats/min, with an average and standard deviation of 112.47±29.36. Statistical tests did not reveal any significant differences between two genders and ECG parameters. On the other hand, there was a significant difference between different age groups in the heart rate ($p<0.001$), P durati-

on ($p=0.030$), QRS duration ($p=0.005$), and the P–R interval ($p=0.005$), Q–T interval ($p<0.001$), and R–R interval ($p<0.001$). Sheep with sinus arrhythmias had a significantly lower mean heart rate than sheep with normal rhythm ($p=0.007$). Furthermore, an analysis indicated that there was a significant difference between the age groups and the cardiac dysrhythmias ($p<0.01$). The results of this study can be used as a reference in studies on the Shall sheep breed.

Keywords: Age, electrocardiogram, gender, parameter, Shall sheep

Introduction

Electrocardiography (ECG) is a non-invasive, easy-to-use diagnostic technique that evaluates the electrical system of the heart to assess cardiac dysrhythmias and conduction abnormalities. It also provides a meaningful reflection of cardiac size, enlargement of chambers, myocardial damages, and metabolic disorders (Constable et al., 2016). Determination of the electrocardiogram normal values allows investigators to distinguish between pathological and physiological cases. A base apex lead has been shown as an ideal lead system for detecting cardiac dysrhythmias in large animals (Cedeno et al., 2016; Kumar et al., 2015; Rezakhani et al., 2004b). The Shall breed, as a dual-purpose sheep breed, originates from the Ghazvin area in central Iran with the population of over 600,000 heads. The Shall sheep is fat tailed, mostly black or brown, with white spots on the head (Hosseini-Zadeh, 2015; Salehi and Taherpour-Dari,

2005). Recently, a number of studies have been carried out to investigate the normal ECG values in different sheep breeds (Chalmeh et al., 2015; Sudhakara and Sivajothi, 2018; Tajik J. et al., 2016). On the other hand, reviewing the literature revealed that no information is available on the normal ECG parameters of clinically healthy Shall sheep breed considering different gender and age groups. Hence, this study was conducted to investigate the ECG values of Shall sheep and to evaluate the effect of gender and different age on the ECG of this breed.

Materials and Methods

This study was carried out on 53 Shall sheep kept at the Aminabad Research Institute of Tehran University (latitude of 35° 34' N and longitude 51° 29' E, 1029 m above the sea level). A proportional stratified random sampling method was used to select the study population. A total of 530 shall sheep were

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selected, and the animals were divided into four groups (240 male sheep, 290 female sheep, 250 lambs, and 280 adults) regarding their gender and age. Ten percent of each stratum was randomly chosen based on the ear tag. No signs of failure in the cardiovascular system (edema, jugular distension, or pulsation) of the sheep were observed. Before obtaining the ECG, the health of animals was confirmed by physical examination. A single-channel electrocardiographic machine (Fukuda 501B-III, Japan) with the paper speed of 25 mm/sec and calibration of 10 mm equal to 1 mV was used. The sheep were held in the restraint box, 10 minutes prior to recording the ECG. To avoid stress reactions of sheep separated from the rest of the flock, the restraint box was placed inside the sheep flock. As much as possible, familiar shepherds were used to carry out the study. The connection between the small alligator electrode clips and the sheep's skin were moistened by 70% isopropyl alcohol. The positive electrode (left arm) was positioned in the fifth left intercostal space at the elbow level near the cardiac apex, the negative electrode (right arm) was attached to the left jugular groove at the cardiac base top, and the ground lead was placed on site away from the heart (Constable et al., 2016). To record the electrocardiograms, the base apex lead, limb lead I was used for at least 2 minutes. To analyze and measure the ECG parameters, a magnifying glass was used. By means of this method, the precision of duration and amplitude was 0.02 s and 0.05 mV, respectively. In the next step, the cardiac rate and rhythm, the amplitude of P, Q, R, S, and T waves; the duration of P, QRS, and T waves; and the P-R, Q-T, and R-R intervals were calculated and recorded. The heart rate was determined by measuring the R-R interval. A statistical analysis was carried out using the Statistical Package for the Social Sciences 20 (SPSS IBM Corp.; Armonk, NY, USA), and the data were expressed as the mean±standard deviation. The independent samples t-tests were used to evaluate statistical differences in the heart rate, wave amplitude, and duration, and the duration of the P-R, Q-T, and R-R intervals between the two genders and the two age groups.

Pearson's correlation test was used to evaluate the relationship between the heart rate and age. Comparison of dysrhythmias between the two genders and age groups were performed using chi-square tests. The findings of this study were considered statistically significant at a p-value <0.05.

Results

The heart rate range was 71–166 beats/min, with an average and standard deviation of 112.47±29.36. There was a strong negative correlation between the heart rate and age ($r=-0.875$, $p<0.001$). The analysis of the ECG waveform and the associated parameters (features) is presented in Tables 1 and 2.

Statistical tests in the studied animals did not reveal any significant differences between the heart rate, amplitude, and duration of ECG parameters and the duration of the P-R, Q-T, and R-R intervals with gender. In contrast, there was a significant difference between different age groups in the heart rate ($p<0.001$), P duration ($p=0.030$), QRS duration ($p=0.005$), P-R interval ($p=0.005$), Q-T interval ($p<0.001$), and R-R interval ($p<0.001$). Further analysis showed that the sheep with sinus arrhythmias had a significantly lower mean heart rate than sheep with normal rhythms ($p=0.007$). A chi-squared analysis indicated that there was a significant difference between the age groups and the dysrhythmias ($p<0.01$, $df=2$, $\chi^2=10.967$). The frequency of cardiac dysrhythmias in different age groups of Shall sheep is shown in the Figure 1.

Sinus tachycardia and sinus arrhythmia were two cardiac irregularities observed in the study. It is interesting to note that the normal cardiac rhythm was not observed in any of the 25 lambs examined in the study. From the Figure 1, it can be seen that the frequency of sinus tachycardia in Group 1 (lambs) is higher than in Group 2 (adults), and the difference is statistically significant ($p<0.001$, $df=1$, $\chi^2=26.323$).

Table 1. Heart rate, amplitude, and duration of the electrocardiographic waves in different genders and age groups of Shall sheep

	All sheep	Male sheep	Female sheep	Group 1 (lambs)	Group 2 (adults)
Number of animals	53	24	29	25	28
Heart rate (Beats/min)	112.47±29.36	117.50±29.11	108.31±29.42	139.40±12.89**	88.42±15.56**
P amplitude	0.152±0.038	0.141±0.038	0.161±0.036	0.160±0.032	0.145±0.042
P duration	0.040±0.007	0.039±0.007	0.041±0.006	0.038±0.007*	0.042±0.006*
P-R interval	0.118±0.027	0.117±0.020	0.119±0.032	0.107±0.013†	0.128±0.033†
QRS amplitude	0.670±0.193	0.676±0.223	0.665±0.169	0.689±0.144	0.653±0.230
QRS duration	0.044±0.011	0.042±0.009	0.045±0.012	0.040±0.005†	0.048±0.013†
Q-T interval	0.262±0.049	0.256±0.045	0.266±0.052	0.222±0.015**	0.297±0.040**
T amplitude	0.431±0.234	0.427±0.198	0.435±0.263	0.456±0.153	0.409±0.289
T duration	0.063±0.025	0.063±0.013	0.062±0.031	0.058±0.013	0.067±0.031
R-R interval	0.569±0.151	0.544±0.146	0.589±0.154	0.433±0.039**	0.690±0.102**

* $p\leq 0.05$; ** $p\leq 0.001$; † $p\leq 0.01$

Table 2. Configuration of the electrocardiographic waves in Shall sheep

ECG configuration	p wave	QRS complex		T wave	
ECG patterns	+	rS	QS	+	+/-
Number	53	19	34	47	6
Percentage	100%	35.84%	64.15%	88.67%	11.32%

ECG: electrocardiogram

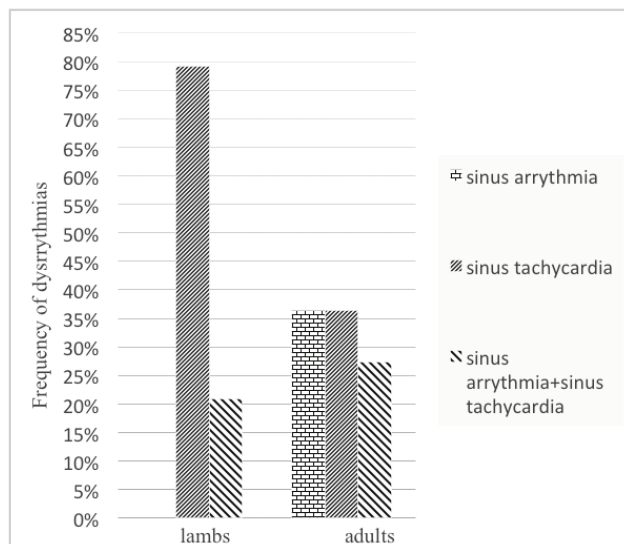


Figure 1. Frequency of dysrhythmias in different age groups of Shall sheep

Discussion

Despite the enormous contribution to human cardiovascular research (Bhatt et al., 2005; Camacho et al., 2016; Milani-Nejad and Janssen, 2014), the studies that have considered normal ECG values and dysrhythmias in sheep are limited. Constant and steady-state configuration in the cardiac parameters, accompanied by negligible effects of animal movement on the ECG quality are the reasons for the use of the base–apex lead system in large animal cardiology (Constable et al., 2016). In reviewing the literature, no data were found on the normal ECG values of the Shall sheep. Hence, the present study was designed to determine the standard values of ECG parameters in clinically healthy Shall sheep based on gender and age.

In our study, the mean heart rate for the Shall sheep was 112.47 ± 29.36 . The mean heart rate was found to be higher in the Kermani sheep with 128.9 beats/minute, and lower in the Balouchi sheep with 89.6 beats/minute, Garol sheep with 85 beats/minute, and fat-tailed sheep with 102 beats/minute (Ahmed and Sanyal, 2008; Rezakhani and Edjtehadi, 1980; Tajik J. et al., 2016; Tajik T. et al., 2016). Previous research has documented that the heart rate can be recognized as an indicator of

age variation in diverse animals (Ferasin et al., 2010; O’connor et al., 2008; Ohmura and Jones, 2017; Rezakhani et al., 2004a; Santarosa et al., 2016), and especially in sheep (Sudhakara and Sivajothi, 2018; Tajik T. et al., 2016). In the current study, the mean heart rate found for adult Shall sheep was 88.42 ± 15.56 , which is statistically significantly lower than the mean heart rate found for lamb Shall sheep (139.40 ± 12.89). There were no significant differences between the two genders and the heart rate. This finding corroborates by Tajik J. (2016) and contrasts with another study (Tajik T. et al., 2016). In this study, the rams had a higher heart rate than the ewes. Certain factors such as animal excitement, age, behavior, and individual identity can affect the heart rate (Baldock et al., 1988; Constable et al., 2016). A significant difference was found between the P-wave duration and age groups of Shall sheep. Nevertheless, this finding is in contradiction with previous reports, which found no apparent difference between the P-wave duration and age groups of different sheep breeds (Chalmeh et al., 2015; Sudhakara and Sivajothi, 2018; Tajik J. et al., 2016; Tajik T. et al., 2016). Also, this result is in agreement with other studies with both human and animal models (Barutçu et al., 2009; Santarosa et al., 2016). The spread of electrical activity through the atria consequently leads to an increase in the heart size with aging. The previous statement matches those observed in earlier studies (Avizeh et al., 2010; Ghadrnan Mashhadi et al., 2016; Reddy and Sivajothi, 2016; Surawicz and Knilans, 2008). Both the PR and QT intervals were longer in adult animals than in lambs, which was statistically significant. The PR interval represents the time impulse transmitted from the sinus node to the atrioventricular node. In addition, the QT interval reflects the ventricular depolarization and repolarization (Muir and Hubbell, 2008; Surawicz and Knilans, 2008). A similar mechanism could explain longer PR and QT intervals in adult animals, as previously described for P duration. The QRS duration was considered a detection criteria in impaired ventricular conduction (Das and Zipes, 2012; Surawicz and Knilans, 2008). Numerous studies have confirmed the age-related changes in QRS duration (Ghadrnan Mashhadi et al., 2016; Mantovani et al., 2013; Rezakhani et al., 2004b). Also, various factors such as hyperkalemia, abnormal or partial impulse formation, and sodium-channel blockers contribute to the QRS complex widening (Muir and Hubbell, 2008; Surawicz and Knilans, 2008). As a hypothesis, it can be argued that in the Shall sheep, the effect of age on the ECG parameters is more prominent than gender. Once again, the reason could be the differences in the size of the heart of the lambs compared to the adult sheep. The influence of gender on ECG parameters has been widely studied (Ghadrnan Mashhadi et al., 2016; Macfarlane, 2018). Furthermore, the effect of sex hormones on ECG parameters has been proven by different authors (Santarosa et al., 2016; Tajik T. et al., 2016; Ziv and Kaufman, 2012). Further studies are required in different sheep breeds to investigate the effects of sex hormones on the ECG parameters.

As shown in Table 2, the configuration of the p waves in all animals were positive. A positive p wave was the most prominent

configuration of p wave in other studies (Rezakhani and Edjtehadi, 1980; Tajik T. et al., 2016; Torío et al., 1997). The dominant QRS configuration in our sheep was QS. The QS morphology has been reported to be the main QRS morphology (in the base-apex lead system) by various researchers (Kamali et al., 2017; Rezakhani and Edjtehadi; 1980, Rezakhani et al., 2004b). The T wave was positive or diphasic (+/-). This finding has been reported by different authors in different sheep breeds (Tajik J. et al., 2016; Tajik T. et al., 2016). There was no normal sinus rhythm in lambs (Figure 1). The impulse initiation and conduction above normal (90 and 120 for adult sheep and lambs, respectively) with origin of the sinus node considered to be sinus tachycardia (Constable et al., 2016). Previous studies have shown that sinus tachycardia is the most prominent arrhythmia in lambs (Chalmeh et al., 2015; Pourjafar et al., 2011). Physiologic sinus tachycardia is mainly rooted in catecholamine, however synergistic inhibition of the vagus nerve plays a role in the formation of this cardiac arrhythmia (Yusuf and Camm, 2005). Sinus tachycardia is frequently associated with excitement, pain, exercise, anemia, hypotension, and the administration of adrenergic agents (Constable et al., 2016; Muir and Hubbell, 2008). As sinus tachycardia is a physiological response to stress, treatment is rarely required (Reed et al., 2018). Another dysrhythmia observed in this study was sinus arrhythmia. The PP interval variability >10% is clearly and unambiguously indicative of sinus arrhythmia (Constable et al., 2016). Physiological reaction to respiration or drug-induced vagal stimulation are predisposing factors for sinus arrhythmia (Das and Zipes, 2012). In the current study, sheep with sinus arrhythmias had a significantly lower mean heart rate than sheep with normal rhythms ($p=0.007$). This finding has been confirmed in other studies (Rezakhani and Edjtehadi, 1980; Rezakhani et al., 2004a; Tajik T. et al., 2016). Cardiac irregularities are considered a normal physiological phenomenon since there were no clinical signs of cardiovascular disease. Finally, results obtained from this study can be used as a reference for future studies, and one of the more significant findings is the suitability of the base-apex lead system in the Shall sheep.

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Antibacterial Efficacy of Some Antiseptics and Disinfectants against Common Bacterial Agents Isolated from Horses in Turkey

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Abstract

Nowadays, many disinfectants and antiseptics are used for decontamination purposes in equine hospitals, on racetracks, and breeding farms, but generally, these antimicrobial agents are not tested against commonly encountered pathogens, and they are used with unknown antimicrobial efficacy. The antimicrobial efficacies of ethanol, chlorhexidine, povidone iodine, sodium hypochloride, peroxymonosulfate compound, and benzalkonium chloride were analyzed using the quantitative suspension test method against the field isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Streptococcus zooepidemicus*, *Streptococcus equi*, *Rhodococcus equi*, and *Staphylococcus aureus*, which are the most frequently encountered pathogens of equines, in the presence of organic load (10% fetal bovine serum) after 1 min, 5 mins, and

30 mins contact times at 20°C. A log reduction of five or more (5 log \leq) in cfu counts of the tested pathogens was considered as effective for each disinfectant and antiseptic. According to the results, except for sodium hypochloride in the 1/100 dilution, all other disinfectants and antiseptics achieved a minimum 5 log reduction and were found to be effective against all tested isolates. Decreased dilutions and/or direct use of the sodium hypochloride should be tested against the same bacterial agents, as well as with multiple field strains. In addition, reference strains of the microorganisms should be evaluated in further studies.

Keywords: Antiseptics, bacterial pathogens, biosecurity, disinfectants, horse

Introduction

Control of infectious diseases in horse populations involves two critical aspects: vaccination and disinfection. Many adequate vaccines against infectious diseases are commercially available, but none of them can be warranted to be 100% effective (Dwyer, 2004). In times of an epidemic disease, it is common to find significant environmental microbial contamination in hospitals, on racetracks, farms, and in any facilities where horses reside. This microbial contamination commonly originates from infected animals' secretions, such as blood, urine, feces, nasal, and conjunctival secretions, etc. (Saklou et al., 2016). It is also important to minimize animal trafficking and distribution of potential pathogens by movement of personnel and fomites

(Morley et al., 2005). Therefore, disinfection and antiseptics management practices are essential parts of providing a healthy environment for horses. Disinfection also plays an important role in the prevention and control of nosocomial infections, especially for the multi-resistant bacteria, where disinfection is the only way to slow down the disease outbreak.

Many bacterial pathogens can cause systemic and local infections in horses. *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) and *S. equi* subsp. *equi* (*S. equi*) cause the lower respiratory tract, joint, genital tract, eye and guttural pouch infections, and abscess formation. In foals, *Rhodococcus equi* (*R. equi*) cause pleuropneumonia, gastrointestinal tract infections, and abscess formation as well. *Staphylococcus aureus* (*S. aureus*)

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may cause wound infections, mastitis, and abscess formation. *Salmonella* spp. are mostly isolated from the gastrointestinal tract infections and neonatal sepsis. *Escherichia coli* (*E. coli*) may cause genital tract infections, mastitis in mares, and septicemia in neonatal foals. *Pseudomonas aeruginosa* (*P. aeruginosa*) cause genital tract infections and mastitis in mares (Sellon and Long, 2013). These bacterial agents can survive on environmental surfaces for long periods with a possible transmission to susceptible hosts. Therefore, it is imperative to use an effective disinfectant/antiseptic to prevent the spread of these agents (Köse and Yapar, 2017).

In field conditions, a good disinfectant should be effective in the presence of organic matter, such as blood, urine, feces, and other body secretions; have a low or zero toxicity against animals; and show the bactericidal activity in a relatively short period of time. Among the horse pathogens, gram-positive and gram-negative bacteria and enveloped viruses are considered to be susceptible to the disinfectants in the absence of organic load. But besides these generalizations, because they are in the same susceptibility category, *Salmonella* species are extremely difficult to eliminate from horse facilities (Dwyer, 2004).

In Turkey, many commercially available antiseptics and disinfectants with different active ingredients are used in equine industry for decontamination of the bacterial agents. But to the best of author's knowledge, no antimicrobial efficacy studies with commercially available antiseptics and disinfectants were performed against the reference and field strains of the horse bacterial pathogens up to this date in Turkey.

The aim of this study was to evaluate the antibacterial effectiveness of disinfectants and antiseptics often used in equine facilities and hospitals, including sodium hypochloride (household bleach), potassium peroxydisulfate (Virkon S; İstanbul, Turkey), and benzalkonium chloride (Quaternary ammonium compound-QAC, Zefirolum; İstanbul, Turkey) as disinfectants as well as ethanol, povidone iodine (Poviodeks; İstanbul, Turkey), and chlorhexidine (Hibitanol; İstanbul, Turkey) as antiseptics

Table 1. Sample type isolated, date of isolation, and geographical isolation region of the bacterial isolates used in the study

Isolate	Sample type isolated	Geographical origin
Gram positive		
<i>S. zooepidemicus</i>	Tracheal wash fluid	İstanbul
<i>S. equi</i>	Guttural pouch wash fluid	İstanbul
<i>S. aureus</i>	Skin wound swab	İstanbul
<i>R. equi</i>	Tracheal wash fluid	İzmit
Gram negative		
<i>E. coli</i>	Intrauterine swab	Thrace
<i>P. aeruginosa</i>	Intrauterine swab	Thrace
<i>Salmonella</i> spp.	Rectal swab	İstanbul

tics against the field isolate of gram-positive species such as *S. zooepidemicus*, *S. equi*, *S. aureus*, and *R. equi*, and gram-negative species such as *P. aeruginosa*, *E. coli*, and *Salmonella* spp. in the presence of organic load to examine the antimicrobial activities of the commercial compound(s) commonly used in horse care facilities and hospitals in Turkey.

Materials and Methods

Bacterial Strains

The field isolates of *S. zooepidemicus*, *S. equi*, *S. aureus*, *R. equi*, *E. coli*, *P. aeruginosa*, and *Salmonella* spp. were used in the study. The isolation side, date of isolation, and isolation region in the country were shown in the table below (Table 1). Briefly, all clinical samples were streaked to 5% sheep blood agar and MacConkey agar, and they were incubated at 37°C in both aerobic and microaerophilic (5% CO₂) conditions for 48 hours. In addition, rectal swab samples were also inoculated in selenite broth for 18 hours and passaged to the XLD agar media for *Salmonella* spp. isolation. Suspected colonies were identified with routine methods, such as colony morphology, microscopic morphology, and gram characteristics, catalase, oxidase, and other biochemical tests using the BBL crystal E/NF and gram-positive identification systems (Becton Dickinson; Sparks, U.S.). After the identification process, the isolates were passaged into tryptone soy broth (TSB) (Oxoid; Basingstoke, UK), containing 20% glycerol, and stored at -20°C until laboratory analysis. The isolates were revived by passaging to the tryptone soy agar (TSA) (Oxoid; Basingstoke, UK) from the storage media.

Disinfectants and Antiseptics

Commercially available three different classes of disinfectants and three different classes of antiseptics were chosen to represent different range of active compounds in the present study. Disinfectant/antiseptic classes, active ingredients, and the dilutions used in the experiment are given in Table 2. Disinfectants and ethanol were diluted using tap water of 207 mg CaCO₃/L hardness in each test tube. The hardness of the tap water was determined with SM 2340:B; ISO 17294-2 (ICP-MS) method by a private environmental analysis laboratory (Çevre Industrial Analysis Laboratory, İstanbul, Turkey).

Culture Media

Tryptone soy broth and TSA were used for maintenance and determination of viable cell counts in the experiment.

Neutralization Media

Neutralization solution was prepared by using a mixture of tryptone (5.0 g/L), yeast extract (2.5 g/L), dextrose (10.0 g/L), sodium thioglycollate (1.0 g/L), sodium thiosulphate (6.0 g/L), sodium bisulphite (2.5 g/L), llecithin (7.0 g/L), polysorbate 80 (5.0 g/L), and bromocresol purple (0.020 g/L). The final pH values of neutralization media were measured and adjusted to 7.6±0.2 before use.

Table 2. Name, class, active ingredients, and dilutions of the disinfectants and antiseptics used in the study

Disinfectant/antiseptic name	Disinfectant/antiseptic class	Active ingredient	Used dilution
Virkon-S	Peroxygen compounds	Potassium peroxy monosulfate (50%)	1:100
Zefirolum	QAC	Benzalkonium Chloride (10%)	1:100
Household bleach	Chlorine compounds	Sodium hypochloride (5.25%)	1:100
Ethanol	Alcohols	Ethanol (70%)	Direct use
Poviodeks	Iodine compounds	Povidone iodine (10%)	Direct use
Hibitanol	Biguanides	Chlorhexidine (4%)	Direct use

Organic Load

Fetal bovine serum (FBS) at a final solution of 10% was used in the experiment.

Contact Time

Contact times of 1 min, 5 mins, and 30 mins at 20°C for each disinfectant/antiseptics against the bacterial suspensions were included in the experiment.

Experiment Control Procedures

Three control procedures were performed to demonstrate the validity of the experiment. Standard tap water that was used in the dilution of the antiseptics and disinfectants was controlled for the lethal effect against bacterial growth. Bacterial growth for all microorganisms tested in the study was determined in TSA after a 24-hour incubation at 37°C. The neutralizan solution used in the study was also checked for the lethal effect on the bacterial growth.

Standard tap water was used in the dilution of the antiseptics/disinfectants and controlled for the lethal effect against bacterial growth. For the evaluation, 1 mL of bacterial suspension and 1 mL of organic substance were added to 8 mL of tap water instead of disinfectant/antiseptic and then incubated for 5 minutes at room temperature. After incubation, 0.1 mL of the incubated suspension was inoculated into TSA and incubated at 37°C for 24 hours and checked for bacterial growth.

The neutralizan solution used in the study was controlled for the lethal effect against bacterial growth. For the evaluation; 8 mL of neutralizan solution and 1 mL of sterile distilled water were added to 1 mL of bacterial suspension and then incubated for 5 minutes at room temperature. After incubation, 0.1 mL of the incubated suspension was inoculated to TSA and incubated at 37°C for 24 hours and checked for bacterial growth.

The effect of the neutralizan solution for each antiseptics/disinfectant used in the study was checked. For control, 1 mL of bacterial suspension and 1 mL of sterile distilled water were added to 8 mL of neutralized disinfectant solution and then incubated for 5 minutes at room temperature. After incubation, 0.1 mL of the incubated suspension was inoculated to TSA and incubated at 37°C for 24 hours and checked for bacterial growth.

Test Method

The effectiveness of the disinfectants was evaluated by the method of quantitative suspension test (Ismail et al., 2015). Broth cultures of bacterial strains were stored at -20°C until the experiments. Cultures were brought to room temperature, and then 0.1 mL of broth cultures were inoculated to TSA, being allowed to grow at 37°C for 24 hours. A subculture was performed for each bacterial strain, and the second subcultures of the bacterial strains were used in the study. All suspensions from the second subcultures were prepared with TSB and adjusted to 1.5x10⁸ cfu (colony forming unit)/mL by plate surface spread viable counting method. The bacterial suspensions were maintained at room temperature and used within 2 hours.

Prior to testing, all reagents were brought to 20°C in water bath. Disinfectant/antiseptics were diluted with tap water as recommended by the manufacturers. 1 mL of FBS solution was added to 8 mL disinfectant/antiseptic solution and mixed by vortexing and left for 30 minutes. 1 mL of bacterial suspension was added to the mixture and inoculated at 20°C for 1 min, 5 mins, and 30 mins, respectively. After contact time of the bacterial strain with the disinfectant/antiseptic solution, 1 mL of disinfectant/antiseptic+bacterial strain mixture was added to 8 mL of neutralization media with 1 mL sterile distilled water and inoculated for 5 minutes at 20°C.

After the neutralization step, 100 µl of mixture was inoculated to the TSA with serial dilutions up to 10⁻⁵ at 37°C for 18 hours to determine cfu counts.

Reduction of viability of the microorganisms were calculated according to the following formula:

$$R = \frac{N \times 10^{-1}}{N_a}$$

where R is the reduction in viability, N is the cfu count of the initial test suspension, and N_a is the cfu count of the mixture at the end of the contact time with the disinfectant/antiseptic suspension.

A minimum log reduction of 5 (5 log_≤) was defined as effective for the disinfectants/antiseptics used in the study.

Table 3. CfU/mL values of *E. coli* after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	1.04x10 ⁵	6x10 ⁴	7x10 ⁰
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

Table 4. CfU/mL values of *P. aeruginosa* after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	5.4x10 ⁴	7x10 ⁴	3x10 ⁴
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

Table 5. CfU/mL values of *Salmonella* spp. after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	8.75x10 ³	2.2x10 ⁴	2.6x10 ⁴
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

Results

Experiment Control

Test results indicated that no antibacterial effect of the neutralizan solution was determined for all tested microorganisms in the study. The neutralizan effect of the neutralizan solution against antiseptics/disinfectants was evaluated for each disinfectant and antiseptic as well. No inhibition in bacterial growth was determined for each neutralized disinfectant and antiseptic used in the study (data not shown).

Antibacterial Activity of the Tested Antiseptics and Disinfectants

According to the standards, antimicrobials tested must show a minimum 5 log (10⁵) reduction in cfu/mL to be considered as effective. After the determined contact times with 70% etha-

Table 6. CfU/mL values of *S. zooepidemicus* after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	4x10 ¹	0	0
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

Table 7. CfU/mL values of *S. equi* after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	2.02x10 ⁴	1.92x10 ⁴	1.17x10 ⁴
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

Table 8. CfU/mL values of *R. equi* after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	2.31x10 ³	1.9x10 ⁴	0
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

nol, chlorhexidine, povidone iodine, virkonS (1/100), and benzalkonium chloride, a 8.17 log reduction was identified against *E. coli*, *P. aeruginosa*, *Salmonella* spp., *S. zooepidemicus*, *S. equi*, *R. equi*, and *S. aureus* in the presence of the organic load (10% FBS). But on the other hand, sodium hypochloride (1/100) failed to pass the test standard against *E. coli* after 1 min and 5 mins; against *P. aeruginosa* after 1 min, 5 mins, and 30 mins; against *Salmonella* spp. after 1 min, 5 mins, and 30 mins; against *S. equi* after 1 min, 5 mins, and 30 mins; against *R. equi* after 1 min and 5 mins; and against *S. aureus* after 1 min and 5 mins contact times in the presence of organic load (10% FBS). The cfu/ml of bacterial agents after 1 min, 5 mins, and 30 mins contact times with the antiseptics and disinfectants are listed as in the tables (Tables 3-9). Results of reduction in viabilities (log reduction) obtained in the present study were given as graph (Figure 1).

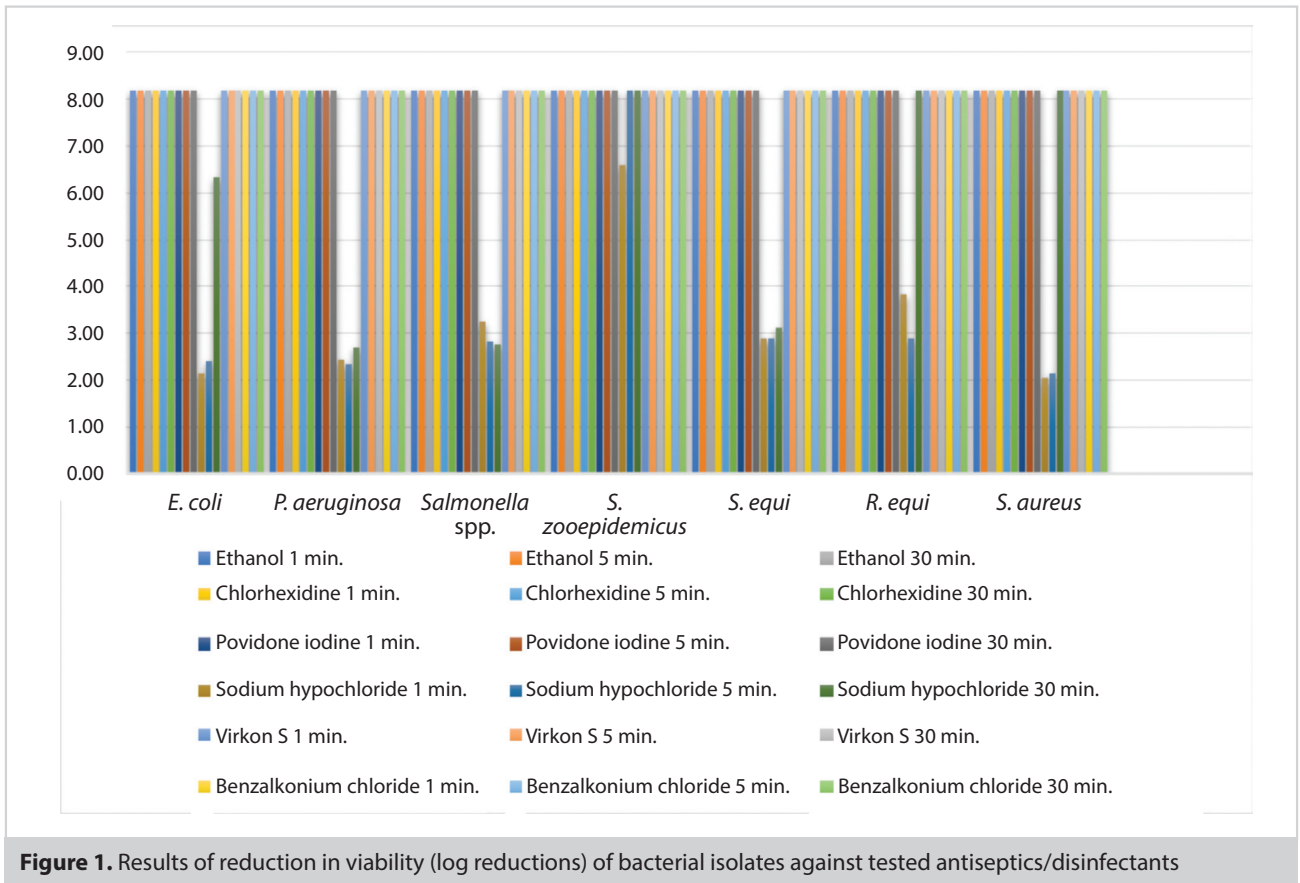


Table 9. Cfu/mL values of *S. aureus* after contact with tested antiseptics and disinfectants at 20°C

Antiseptics-disinfectants/contact time	1 min	5 mins	30 mins
Ethanol	0	0	0
Chlorhexidine	0	0	0
Povidone iodine	0	0	0
Sodium hypochloride	1.39x10 ⁵	1.11x10 ⁵	0
Virkon S	0	0	0
Benzalkonium Chloride	0	0	0

Discussion

The use of disinfectants and antiseptics is of paramount importance in biosecurity and infection control in individuals and populations. Proper use of disinfectants and antiseptics could be expected to be cheaper than economic cost of antimicrobial treatment in horses or loss of part or all of that horse population due to a disease outbreak (Dwyer, 1995). Microorganisms are known to vary in their susceptibility against disinfectants and antiseptics, and some studies reveal that the efficacy of disinfectants are gradually reduced (Orji, 2014). Inappropriate consumption, inaccurate concentration, and lack of training for

preparation and storage are the most common reasons of increasing resistance to disinfectants (Zareniya et al., 2017).

Karayıldırım and Çelenk (2016) expressed that 20% benzalkonium chloride was found to be effective against *E. coli*, *S. aureus*, and *P. aeruginosa* with a 1 min contact time. In the present study, it was determined that the 1/100 dilution of 10% benzalkonium chloride was also effective (log 5 ≤ reduction) in 1 min, 5 mins, and 30 mins against the same bacteria that were isolated from clinical cases of horses. Gehan et al. (2009) indicated that 1% of benzalkonium chloride was effective against *P. aeruginosa*, *E. coli*, *S. typhimurium*, and *S. aureus* at the 30 min contact time. In another study, 3% of benzalkonium chloride achieved a 5 log reduction in 30 mins, and 1% in 60 mins (El Aal et al., 2008). Fazlara and Ekhtelat (2012) described that *Listeria monocytogenes* was the most susceptible bacteria to benzalkonium chloride, followed by *S. aureus* and *E. coli*, respectively, according to the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test results. Considering all these results, in addition to 20% after 1 min of contact time, 1% and 3% concentration of benzalkonium chloride after 30 mins of contact time, and 1/100 dilution of %10 benzalkonium chloride can be used for inactivating *E. coli*, *S. aureus*, and *P. aeruginosa* after 1 min, 5 min, and 30 mins contact time.

Saklou et al. (2016) determined that the mist application of 2% of peroxymonosulfate compound disinfectant at 30 mins contact time created a 84%, 99%, and 99% reduction against *S. enterica*, *P. aeruginosa*, and *S. aureus*, respectively, and it was found to be effective if used after cleaning of the surfaces. Gehan et al. (2009) found that 1% peroxymonosulfate compound was effective against *P. aeruginosa*, *E. coli*, *S. typhimurium*, and *S. aureus* after 30 mins of contact time in the presence of organic matter. Chima et al. (2013) also declared that peroxymonosulfate compound was 100% effective against *Salmonella* spp., *E. coli*, *Klebsiella* spp., and *P. aeruginosa*. Another study also yielded that 1/100 dilution of 50% peroxymonosulfate compound demonstrated 5 log \leq reduction against *S. typhimurium* ATCC 13311 strain (Jang et al., 2017). These results were in concordance with the present study's results: 50% peroxymonosulfate disinfectant in 1/100 dilution showed a 8.17 log reduction at 1 min, 5 mins, and 30 mins, and it was found to be effective (5 log \leq) for all pathogens that participated in the study and found to be effective against the same bacterial pathogens of the previous studies. The present study's results for the peroxymonosulfate compound have been also confirmed in previous studies.

In the present study, 5.25% sodium hypochloride in 1/100 dilution failed to create the 5 log \leq reduction against *P. aeruginosa* at 1 min, 5 mins, 30 mins and *S. aureus* at 1 min and 5 mins of contact time. But contrary to the present study, 5% sodium chloride in 1/100 dilution showed 7.22 and 8.11 log reductions at 5 mins and 30 mins, respectively, in a previous study (Bhosale, 2017). The discrepancy might have been due to the difference of the antimicrobial resistancy of bacterial strains tested in the studies. On the other hand, Addie et al. (2015) specified that household bleach produced a 5 log \leq reduction in *E. coli* 0157:H7 and *Salmonella typhimurium* after 1 min contact time. In the present study, the household bleach was used in 1/100 dilution so that the efficacy might have been reduced due to the dilution factor. In parallel, Avci and Otkun (2017) claimed that the 1/100 dilution of sodium chloride was ineffective against *S. aureus* and *P. aeruginosa* after 1 min and 2 mins contact time but effective after 5 mins, 10 mins, and 30 mins contact time. According to the author, further studies should be designed to test more concentrated dilutions or direct use of the sodium hypochloride against the same pathogens in to test the antimicrobial efficacy. It was also claimed that sodium hypochloride was inactivated by organic debris (Addie et al., 2015). Another reason for reduction in the efficacy of sodium hypochloride against the tested pathogens might have been the interaction with organic material (10% FBS) in the present experiment.

Zareniya et al. (2017) determined that povidone iodine was more effective than 70% ethanol in 49 *P. aeruginosa* isolates according to MIC and MBC values. In the present study both antiseptics demonstrated a full reduction (8.17 log) in *P. aeruginosa*. In another study, 10% povidone iodine was found to be effective after 1 min contact time against *S. aureus*, *P. aeruginosa*,

and *E. coli* (Avci and Otkun, 2017). In the same study 70% ethanol was effective against *P. aeruginosa* and *E. coli*, but just ineffective against *S. aureus* after 1 min contact time. After 2 mins, 5 mins, 10 mins, and 30 mins, 70% ethanol was found to be effective by Avci and Otkun (2017). The present study revealed that 70% ethanol and 10% povidone iodine demonstrated efficacy (5 log \leq) against *P. aeruginosa*, *S. aureus*, and *E. coli* after 1 mins, 5 mins, and 30 mins contact time, and the results were mostly in concordance with Avci and Otkun's (2017) results. According to the past and present study results, 70% ethanol and 10% povidone iodine can be used for antiseptics and disinfection purposes. The present study has some limitations, such as limited number of field bacterial isolates were tested, and a single test method (quantative suspension test) was used. Therefore, future studies should include more of field strains and evaluate different efficacy methods to monitor the antimicrobial activity of the tested disinfectants.

In conclusion, 50% peroxymonosulfate compound in 1/100 dilution; 10% benzalkonium chloride in 1/100 dilution as disinfectants; and 70% ethanol, 4% chlorhexidine, and 10% povidone iodine as antiseptics may be used in equine hospitals and equine care facilities as decontaminating agents against *E. coli*, *P. aeruginosa*, *Salmonella* spp., *S. zooepidemicus*, *S. equi*, *R. equi*, and *S. aureus* after 1 min, 5 mins, and 30 mins contact time. Sodium hypochloride in 1/100 dilution did not yield satisfactory results, and it failed to achieve a 5-log reduction against most of the bacterial agents tested in the study. The dilution ratio of sodium hypochloride may be decreased or used without diluting while testing against the bacterial agents in further studies. The present study tested one field isolate of each bacterial species against disinfectants and antiseptics as representatives. Future comprehensive studies should also be performed with multiple field and reference strains of *E. coli*, *P. aeruginosa*, *Salmonella* spp., *S. zooepidemicus*, *S. equi*, *R. equi*, and *S. aureus*, as well as other bacterial and mycotic pathogens with different analytical methods to evaluate the antimicrobial efficacies of the disinfectants and antiseptics.

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Aytekin Günlü	Hüseyin Yılmaz	řerife řule Cengiz
Burhan etinkaya	Hüsnü Erbay Bardakçiođlu	řükrü Kırcan
ađla Parkan Yaramıř	Jie Li	Taci Cangül
Deđer Toplu Oral	Kemal Ak	Trpe Ristoski
Dođukan Özen	Mahmoud Dawood	Uđur Uslu
Ekaterini Theodosiadou	Mehmet Tolga Tan	Ulař Acaröz
Eleni Tzika	Muammer Göncüođlu	Ümüt Cirit
Elias Papadopoulos	Mustafa Saatçi	Yavuz Nak
Elif Ergül Ekiz	Mustafa Uđurlu	Yılmaz Aral
Elmutaz Atta Awad	Müjdat Alp	Yusuf Ođrak
Erdal Matur	Neře Kocabađlı	Zekeriya Nur
Erhan Keyvan	Olarinke Victoria Adeniyi	Zoi Polizopoulou
Funda Yıldırım	Omolade Oladele	
George C. Fthenakis	Ömür Koçak	