

**Searching Bovine Herpesvirus Types 1, 4 and Bovine Parainfluenza Virus Type 3 Infections in Milk of Cows with Clinical Mastitis Based in Dairy Cattle Managements<sup>†</sup>**

**ABSTRACT**

In 35 managements around Burdur region, milk of 123 cows with clinical mastitis was searched for Bovine Herpesvirus Types 1 (BHV-1), 4 (BHV-4) and Bovine Parainfluenza Virus Type 3 (BPIV-3) infections. In the study, the highest seropositivity was detected against BPIV-3. The highest seropositivity on infection distribution according to age was found against each four viruses in animals within the three-year-old group. The highest seropositivity in this group and other age groups was detected against BPIV-3. Seropositivity against these viruses was found in the highest right anterior one and the lowest left posterior one out of determined udder lobes. The highest seropositivity was found in semi-outdoor managements with concrete and dirty grounds where cleaning/disinfection of teats before and after milking was performed, mastitis treatment and viral vaccination was not applied, the ground of stable was cleaned monthly, only feces was collected from the grounds, water was used for cleaning of milking machines and hands and iodised disinfectant was used. During udder inspection evaluation of animals with clinical mastitis, the highest seropositivity was detected in the ones having normal udder, teat and skin appearance. Out of udder and teat skin lesions, the highest seropositivity was found in crushed ones. In the evaluation of udder palpation in animals with clinical mastitis, the highest seropositivity was detected in the ones with teat tissue thickening and with elastic teat sinuses and lobes. In the milk of these animals, the highest seropositivity was found in the ones showing coagulation. Consequently, it was stated that viruses took a vital part in clinical mastitis cases, the structure, practice, cleaning and disinfection of managements was really important, udder and teat skin lesions, tissue thickening, elastic teat sinuses and lobes and coagulation of milk was so crucial. Besides, the appearance of udder, teat and skin did not matter.

**Keywords:** Cow; clinical mastitis; milk, viruses.

## 1.INTRODUCTION

Mastitis is defined as the inflammation developing in parenchyma tissue of one or more udder lobes resulting from toxic, traumatic or infectious factors. Characteristic feature of mastitis is characterized by pathological disorders in mammary gland and physical, chemical and frequent bacteriological changes in milk [28].

In many studies to detect etiology in mastitis cases, no etiological agent could be detected in 20-35% of the cases [25,40]. In this case, researchers claimed that there could be factors in small quantities or hardly isolated agents such as fungi, yeast or chlamydia except bacteria could be possible. Watts [40], who detected 137 various microorganisms causing mastitis, did not include viral etiology in his study. The virus infections within bovine clinical and subclinical mastitis were reported as Bovine herpesvirus type 1 and type 4 (BHV-1 and BHV-4), Bovine Parainfluenza type 3 virus (BPIV-3), Foot and mouth disease virus (FMDV) and Bovine leukemia virus (BLV) [20,33,41,45,46]. Besides, Bovine herpesvirus type 2 (BHV-2), Bovine vaccinia virus (BVV), Cowpox virus, Pseudocowpox virus, Bovine vesicular stomatitis virus (BVSV), Bovine papillomavirus (BPV), Bovine viral diarrhea virus (BVDV), Bovine immunodeficiency virus (BIV) and Rinderpest virus (RPV) were stated to have joined bovine mastitis indirectly [23,30,31,36,37,39,43,44].

## **2.MATERIALS AND METHODS**

### **2.1.Animals and Properties of Managements**

In this study, milk samples were collected from four udder lobes of each of 123 Holstein dairy cattle over three years old with clinical mastitis. Animals with blind teats were not included in the study. Samplings were collected from animals whose BHV-1 and BPIV-3 vaccinations were not applied. Because constant vaccination procedure (2-3 times a year) against FMDV disease is applied for all dairy cattle for protection in our country, this was not included in our search study in terms of viral agent (for vaccination based positivity would be detected). For pre-diagnosis of clinical mastitis, methods of udder inspection and palpation and searching milk appearance (physical examination) were used. In the study, systemic symptoms that might be seen in cows with mastitis (fever, increase in pulse and respiration, inflammatory symptoms in mammary gland, weakness, lack of appetite, pause in rumen movements etc.) were not taken into consideration. A total of 35 managements were searched. Questions and replies about properties of animals and managements were recorded.

### **2.2.Udder Inspection**

Sizes of udder parts and teats (in proportion to one another), shape of udder (droopy, skewed), appearance of teat and skin (strained, crumpled, colour changes), udder and teat skin lesions (ragged, crushed, cut wounds, crustation, necrosis, fistule, ulcer, oedema) were evaluated.

### **2.3.Udder Palpation**

Evaluations of teat tissue thickening in terms of fibrosis aspect, controlling of udder sinuses (condition of softness and elasticity) and palpation of udder lobes (modal form, condition of softness and elasticity) were performed.

#### **2.4. Searching the Appearance of Milk**

Colour change, dilution, smell, flacon or coagulation was searched in collected milk. A black, flat ground was used in these examinations.

#### **2.5. Milk Samplings**

Milk samples were taken into 10 mL sterile glass tubes<sup>1</sup> from each udder lobe of animals having clinical mastitis. Collected milk samples were transferred into laboratory environment in cold chain.

#### **2.6. Collecting Milk Serum**

After adding 0.2 mL rennin<sup>2</sup> and 0.1 mL saturated  $\text{CaCl}_2$ <sup>2</sup> onto milk samples taken as 10 mL into sterile glass tubes<sup>1</sup>, it was left for incubation for 1 h at 37°C. Later it was centrifuged for 20 min at 720 g and the cream layer was removed by the help of a spatula. Milk serum was collected using a pasteur pipette and was inactivated in water bath for 30 min at 56°C before taken into deep freezer. Milk serum was kept in a -40°C deep freezer until applying ELISA test.

#### **2.7. BHV-1, BHV-4 and BPIV-3 ELISA (milk)**

In the milk serum samples taken from animals in managements, ELISA BHV-1<sup>3</sup> seroconversion detection commercial testing product was used to detect antibody presence against BHV-1, ELISA BHV-4<sup>4</sup> antibody detection against BHV-4 and ELISA BPIV-3<sup>5</sup> seroconversion detection against BPIV-3. Applications were performed according to kit procedures. All the plates were read under ELISA reader<sup>6</sup>. The obtained positive, negative and optical density results of samples were evaluated according to kit procedure. Validation of all tests was confirmed before the evaluation.

### **3.RESULTS**

#### **3.1.Animals and Properties of Management**

Surveys with breeders were applied in managements where seropositivity was detected. Animals and managements where no vaccination was performed against factors such as BHV-1 and BPIV-3 were studied. Out of samples taken separately from four teat lobes of each animal, seropositivity against viruses was detected in only one animal's four teat lobes. Within 35 managements, average seropositivity rate was found as 35.4% for right front teats, 30.2% for right rear teats, 21.8% for left front teats and 12.6% for left rear teats, average seropositivity distribution according to stable properties was found as 30.8% for indoor ones, 21.3% for outdoor ones and 47.9% for semi-outdoor ones, average seropositivity distribution according to stable location was detected as 65.3% for concrete ones, 10.2% for inlay ones and 24.5% for soil ones, average seropositivity distribution according to cleanness of stable ground was found as 82.4% for dirty ones and 17.6% for clean ones, and because milking was performed by machines in all managements, average seropositivity distribution was detected as 100%. Average seropositivity distribution was found as 71.4% in managements where teat cleaning/disinfection was applied before and after milking and 28.6% where not applied, 42.8% where mastitis treatment was applied previously and 57.2% where not applied, 12.4% where stable ground cleaning was performed daily, 28.3% where performed weekly and 59.3% where performed monthly, 29% where stable ground was cleaned with water, 8.7% with disinfectants and 62.3% only by collecting feces, 80.9% where milking machines were cleaned with water, 16.4% with disinfectants and 2.7% with no cleaning, 92% where hands were cleaned with water, 1.4% with disinfectants and 6.6% with no cleaning, and 97.8% where iodised disinfectant was

used and 2.2% where not used. The distribution of sampled animals according to ages was shown in Table.1. The distribution of infection according to ages was shown in Table.2.

**Table 1. The distribution of sampled animals according to ages**

<b>Ages (year)</b>	<b>Numbers of animal (n, %)</b>
3	56 (45.5%)
4	27 (22%)
5	17 (13.8%)
6	11 (8.9%)
7	6 (4.9%)
8	5 (4.1%)
9	1 (0.8%)
<b>Total</b>	<b>123 (100%)</b>

**Table 2. The distribution of infection according to ages.**

<b>Ages (year)</b>	<b>BHV-1 (n, %)</b>	<b>BHV-4 (n, %)</b>	<b>BPIV-3 (n, %)</b>
3	44 (35.8%)	37 (30.1%)	56 (45.5%)
4	15 (12.2%)	15 (12.2%)	27 (22%)
5	10 (8.1%)	8 (6.5%)	17 (13.8%)
6	5 (4.1%)	5 (4.1%)	11 (8.9%)

7	2 (1.6%)	1 (0.8%)	6 (4.9%)
8	2 (1.6%)	1 (0.8%)	5 (4.1%)
9	1 (0.8%)	1 (0.8%)	1 (0.8%)
<b>Total</b>	<b>79 (64.2%)</b>	<b>68 (55.3%)</b>	<b>123 (100%)</b>

### 3.2.Udder Inspection

Average seropositivity distribution was detected as 62.5% for animals with normal udder shape, 25% for droopy ones and 12.5% for skewed ones, 60% for animals having normal appearance of teat and skin, 10% for tight ones and 30% for crumpled ones. However, no colour change was observed. Average seropositivity distribution on udder and teat lesions was found as 27.9% for crushed ones, 15.8% for ragged ones, 6.3% for cut wounds, 18.5% for oedema, 7.7% for crustations and 2.5% for fistules. No skin lesion was observed for 21.3%.

### 3.3.Udder Palpation

Average seropositivity distribution was found as 7.7% for tissue thickening in teats while it was 92.3% in teats with no thickening. Fibrosis formations were not observed in teats. Seropositivity was found as 26.2% for animals with soft udder sinuses and lobes and 73.8% with elastic ones.

### 3.4.Searching the Appearance of Milk

In studies applied in terms of appearance of milk, average seropositivity distribution was detected as 10.4% for diluted ones, 78.4% for coagulated ones and 11.2% for the ones with colour change. Distribution of seropositivity in single, double and triple infection was shown in Table.3.

**Table 3. Distribution of seropositivity in single, double and triple infection results**

<b>Viruses</b>	<b>Seropositivity rates (n, %)</b>
BHV-1	79 (64.2%)
BHV-4	68 (55.3%)
BPIV-3	123 (100%)
BHV-1 + BPIV-3	15 (12.2%)
BHV-4 + BPIV-3	9 (7.3%)
BHV-1 + BHV-4 + BPIV-3	39 (31.7%)

#### 4.DISCUSSION

Cow milk constitutes 83% of total world milk production. 54% of world cow and buffalo milk takes place in Asia and Europe continents. On the basis of countries, some particular regions have a critical importance in terms of milk production. According to data in 2014, Turkey is one of the rare countries with an annual growth rate of more than 3% in terms of relevant milk production. Turkey was reported to have produced 16 million tonnes of milk in 2014 [26].

Microorganism groups are the primary reason of cattle mastitis. Microorganisms causing cattle mastitis are *staphylococcus*, *streptococcus*, gram negative bacteria, types of mycoplasma, chlamydia, *Arcanobacterium pyogenes*, fungi, mould, ferments and viruses [1]. Not many studies were performed especially on viruses.

In this study, BHV-1, BHV-4 and BPIV-3 infections were studied in the milk of 123 cows with clinical mastitis in 35 managements around Burdur region. Seropositivity against BHV-1 was detected in 64.2% (79/123) of milk samples. Karaduman and Gur [20] studied the role of BHV-1 infection in subclinical and clinical mastitis cases in dairy managements. In their study, they diagnosed 15 cows with clinical mastitis and detected BHV-1 seropositivity in 8 (53.3%) of them. They also found the infection rate as 40.9% for the ones with subclinical mastitis. Siegler et al. [34] reported that mastitis incidence was higher than normal in herds where BHV-1 and BVDV infections were seen together. Besides, in herds taken under control in terms of BHV-1 and BVDV, mastitis rates were found low despite the existence of mastitis caused by *staphylococcus* and *streptococcus*. Some researchers [9-



11] reported that the virus replicated in mammary gland when BHV-1 was inoculated inside teats and that clinical mastitis developed. In teat lobes where the virus was inoculated, swelling, sensitivity and stiffening was found as symptoms, milk composition was collapsed and milk yield decreased considerably. Unlike these situations, Herlekar et al. [15] studied BHV-1, BHV-2, BHV-4 and BVDV presence in milk samples by real-time PCR they developed. They found BHV-1 seropositivity in 10% of milk samples they collected from cows. Bilge [7] performed BHV-1 isolation in only one milk sample out of 96 cows with mastitis. Wellenberg et al. [41] could not detect any BHV-1 presence out of 58 clinical mastitis cases naturally obtained in ten herds.

In the study, BHV-4 seropositive animals were determined at a level of %55.3 (68/123). Ali et al. [2] studied BHV-4 presence in the milk of cows with clinical or subclinical mastitis. In their study, they searched for BHV-4 presence in 176 milk samples using ELISA (antibody), PCR and virus isolation tests. In the study, they found antibody presence in 173 of the samples (98.2%) and viral genome presence in 2 (1.3%). However, no virus isolation was performed in two samples where BHV-4 viral genome presence was detected. Wellenberg et al. [41] performed the first isolation of BHV-4 on the milk samples of cows with clinical mastitis. They performed BHV-4 isolation on the milk of three cows in three different herds. They also reported that antibody against BHV-4 developed in 16% of cows with mastitis and 10% of control groups. Herlekar et al. [15] studied BHV-1, BHV-2, BHV-4 and BVDV presence in milk samples by real-time PCR they developed. They found BHV-4 seropositivity in 0.7 % of milk samples they collected from cows.

In the study, the highest seropositivity was detected against BPIV-3. This rate was found as 100% (123/123). Kawakami et al. [21] detected respiratory problems, fever, weight loss, lumps and stiffness in tit lobes, change in milk color and increases in milk pH, mammary gland epithel cells, neutrophils, lymphocytes and monocytes in cattle as a result of in-mammary inoculations they performed experimentally with BPIV-3. After the 10th day of inoculation to all tits, they found high titer ( $>10^7$  DKID<sub>50</sub>/0.1 ml) virus presence in milk. They observed interstitial inflammation and dense lymphoid cells in their histological studies. Kawakami et al. [21] stated that teat tissues were sensitive to BPIV-3 virus and mastitis might develop in naturally infected cows with this virus. Woods et al. [45] searched viral agents in mammary lymph nodes of 42 Hereford cows with low reproductive performance. They detected BPIV-3 virus seropositivity in all blood serum samples of these animals. Valarcher and

Hagglund [38] found the BPIV-3 seropositivity rate as 100% in blood samples of cattle in Southern and Central France.

Although antibody against BHV-1, BHV-4 and BPIV-3 was detected in milk samples of animals with clinical mastitis in this study, antibody that appeared due to systemic infections because of inflammation within the udder in mastitis might also be transmitted to milk. Therefore, we believe that this antibody obtained from milk might be related not only with a udder infection but also a systemic infection.

BPIV-3 seropositivity was mostly seen in double and triple mixed virus infections. BPIV-3 is the virus which is common among adult cattle all around the world and has the highest seroprevalance [8]. It was stated that BPIV-3 virus was quite sensitive to mammary glands, teats could be infected in natural BPIV-3 virus infections and end up with clinical mastitis cases [42]. In this study, the highest seropositivity in infection distribution according to age was found against each three viruses in animals of three-year-old age groups. In this group and other age groups, the highest seropositivity was detected against BPIV-3. Age is a crucial preparatory factor in mastitis. In some studies, risk of mastitis is said to have increased with age increase [6]. This condition, depending on age increase, is associated with the increase in teat gap, decrease in local defense mechanism, continuous birth and the increase in environmental bacterial contamination in birth [14,27]. Besides, cows were under stress and their immune systems got weaker in multi-births. Generally, immunity might get weak and cause mastitis in old animals [14]. In our study, no comments were available since number of animals in age groups were not the same but different.

Out of detected udder lobes, seropositivity against at least one of each three viruses was found in the highest right front and the lowest left behind ones. The highest seropositivity was detected in semi-outdoor, concrete ground and dirty ground managements where teat cleaning/disinfection was applied before and after milking, mastitis treatment and viral vaccination was not applied, stable ground cleaning was performed monthly, only feces was collected from the ground, milking machines and hands were cleaned with water and iodised disinfectant was used. In managements with high mastitis prevalence, low hygiene conditions and low milking hygiene applications existed [24]. As a result, during milking, pathogens might be contaminated and spread around [22]. Çetin and Alan [12] encountered mastitis cases in 125 right front, 113 right rear, 116 left front and 125 left rear udder lobes of cows. Ali et al. [3] found clinical mastitis cases in right front mammary lobes of buffaloes at a rate of

19.29%, 26.32% in right rear mammary lobes, 12.28% in left front mammary lobes and 34.21% in left rear mammary lobes.

As Biffa et al. [6] studied the frequency of clinical mastitis cases according to conditions of breeding, they detected it as 4.6% in outdoor systems, 13% in indoor systems and 13.1% in semi-outdoor systems. Rahman et al. [29] detected mastitis cases in managements with block brick ground as 30% in dry seasons, 58.5% in wet seasons, and in managements with soil ground as 20% in dry seasons, 28.6% in wet seasons. In our study, we found an average seropositivity as 65.3% on concrete grounds and 24.5% on soil grounds. Mekibib et al. [24] detected positivity as 65% on muddy soil stable grounds and 52% on poorly-built stable grounds. In a study [29], stable ground was stated as a crucial factor in mastitis formation. Especially because soil grounds dried much quicker compared to concrete grounds, fewer mastitis cases existed [16]. This condition is connected with the fact that stable ground creates an important potential source for mastitis factors entering into udder through teats. In wet seasons with intense amounts of water, stable ground creates a potential risk [22]. Muddy and non-drained stable grounds were stated to have prepared a suitable environment for microorganisms to grow as a result of hot weather and increasing level of moisture [13]. Using ground sills for cows and their types is crucial for udder health, milk quality and podiatry. For mastitis, both the type of sill (stalk, sawdust, wood powder, sand, ash, chopped paper, straw and mattress) and its volume and replacement frequency is important [4]. California Mastitis Test (CMT) positivity was found as 42.1% in managements where teat cleaning before and after milking was performed with water and drying was applied, 54.3% in managements where cleaning was performed only with water and 62.5% in managements where no cleaning was done [24]. Researchers Bedacha and Menghistu [5] stated the rate of those performing disinfection and hand cleaning before milking as 73.8% while it was 26.2% for those that did not apply these. Besides, the same researchers [5] found the rate of disinfection and washing udders and teats before milking as 21.4% while it was 78.6% for those that did not apply these. Persistence in teat cleaning, suitable stable grounds and their regular cleaning, personal cleaning of milking people, regular teat-dipping applications in milking, fast treatment of clinical cases and diagnosing subclinical mastitis factors were said to cause mastitis prevalence to decrease [29].

During udder inspection evaluations of animals with clinical mastitis, the highest seropositivity was detected in the ones with normal udder, teat and skin appearance. Out of udder and teat skin lesions, the highest seropositivity was detected in crushed ones. During teat palpation evaluations of animals

with clinical mastitis, the highest seropositivity was detected in the ones with tissue thickening in teats, and in the ones with elastic teats and lobes. In the milk of these animals, the highest seropositivity was found in coagulated ones. During applications of milking through microorganism entrance via mammary duct from udders and teats and sucking of youngsters, contamination from cow to cow or among udder lobes might take place depending on milking systems [14]. Mekibib et al. [24] reported that they encountered the highest mastitis prevalence (85.7%) in udder and teat injuries. In udder and teat open injuries, clinical mastitis cases (25.2%) were reported to be higher than those without injuries (5.4%) [6]. Besides, they stated that mastitis cases existed in 68.8% of udder and teat injuries and in 18.2% of the ones without injuries [6]. Hussain et al. [18] studied udder and teat lesions of animals detected as mastitis positive for buffaloes. Accordingly, they found teat lesions as 69.57%, skin rashes as 60%, inflammations 65.22%, rope formations as 33.33%, haemorrhage as 60%, necrosis as 50%, udder oedema as 60% and normal facts as 38.46%. Slettbakk et al. [35] observed the closeness of teat to the bottom, periparturient oedema and teat skewness in animals with clinical mastitis. A relation is considered to exist between mastitis and teat size, general shape of teats, teat lesions, teat pigmentation and milk viscosity [17] while no consensus was created at the level of books and articles [32].

## **5.CONCLUSIONS**

In this study, viruses were thought to take place significantly or viruses were encountered in cases of clinical mastitis. They were found especially as single and triple. The highest seropositivity was detected against BPIV-3 in three-year-old and other age groups.

The physical structure of the management, applications of milking-vaccination-treatment and all kinds of cleaning and disinfection was found effective in clinical mastitis development. Besides, udder and teat skin lesions, tissue thickening, elastic udder sinuses and lobes and coagulation of milk was also in the foreground to cause this condition. However, udder shape and teat skin appearance was not considered important in clinical mastitis cases.

Mastitis is the most crucial matter in dairy cattle breeding all over the world [19]. In order to overcome this matter, studies need to be performed as well in detecting factors in terms of viral agents other than classical approach. By performing experimental and field aimed studies on mastitis cases with viruses and other microorganisms, new data must be obtained. In the light of this data, protective

strategies and methods must be developed. Herd vaccinations must be applied in order to prevent viral mastitis cases. Individuals must be careful, attentive and sanitized about physical structures of managements, applications on herd health and management and all kinds of applications performed on animals individually.

## **MANUFACTURERS**

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