Acta Scientiae Veterinariae, 2017. 45: 1478.

RESEARCH ARTICLE
Pub. 1478

ISSN 1679-9216

Detecting the Effect of Umckaloabo/EPs®7630 Liquid Extract after its Therapeutic Purposed Usage in Calves Showing Symptoms of Respiratory Tract Infection

Ahmet Ak & Mehmet Kale

ABSTRACT

***Background*:** Respiratory tract diseases are commonly seen in beef cattle. Young calves are affected with many respiratory pathogens. Viral pathogens are particularly seen. There are many causative factors, e.g. environmental conditions, immune system of calves. Therefore, alternative treatments are needed for viral respiratory infections. The purpose of the current study was to investigate effectiveness of Umckaloabo/EPs®7630 liquid extract in some bovine viral pathogens of young beef calves.

***Materials, Methods & Results*:** Antibody presence in terms of bovine herpesvirus type 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine parainfluenza virus type 3 (BPIV-3), bovine respiratory syncytial virus (BRSV) and bovine adenovirus type 3 (BAV-3) was searched in blood serum samples of 40 Holstein calves aged 6 months and over showing respiratory tract infection symptoms. All animals were found seronegative in terms of other factors except BRSV. Out of 20 BRSV seropositive calves, 10 of them were classified as control group and the other ten as testing group. BRSV antibody titers were also detected in blood samples of both groups on day 0. Umckaloabo/EPs®7630 liquid extract was given through oral route to animals in testing group according to their weights for 14 days morning, noon and night. No application was performed on animals in control group. BRSV antibody titers were detected in blood samples of animals in both groups taken on days 1st, 3rd, 5th, 7th, 10th and 14th. At the end of day 14th, BRSV antibody titer increased in 9 out of 10 animals (90%) in testing group that were given Umckaloabo/EPs® 7630 liquid extract while one of them (10%) showed no variability. BRSV antibody titer increased in 6 out of 10 animals (60%) in control group while it decreased in one of them (10%) and 3 of them (30%) showed no variability. In testing group, BRSV antibody titer started to increase on day 3rd in 6 out of 10 animals (60%), on day 5th in one (10%), on day 10th in one (10%) and on day 14th in another (10%). In control group, BRSV antibody titer started to increase on day 3rd in 3 out of 10 animals (30%) and on day 5th in 3 of them (30%). When haematologic values of blood samples taken from animals in testing and control groups on day 14th were studied comparatively, no statistical importance ($P < 0.01$ or $P < 0.05$) and difference was detected. As a result, in stock calves showing respiratory tract infection symptoms, applying Umckaloabo/EPs® 7630 liquid extract helped BRSV antibody titer to increase and symptoms decreased. The increase in antibody titers started from day 3rd especially after applying Umckaloabo/EPs® 7630 liquid extract.

***Discussion*:** Respiratory system infections are contagious and fatal in calves. Respiratory tract bacterial and viral vaccinations are applied in order to prevent losses of calves due to these problems. However, these vaccinations are not sufficient most of the time and antibiotics+supportive treatment applications follow afterwards. Therefore, we advise to use a herbal product, Umckaloabo/ EPs®7630 liquid extract, that has both an antiviral efficiency and a feature of supporting immune system in order to prevent mortalities in stock calves, to stop the infection from spreading on all the herd and to decrease the symptoms of respiratory system. We believe that the obtained data should be supported with more examples and more wide-ranging studies.

Keywords: respiratory infection, BRSV, Umckaloabo/ EPs®7630, *Pelargonium sidoides*, calf.

Received: 22 April 2017 Accepted: 20 August 2017 Published: 12 September 2017

Department of Virology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey. CORRESPONDENCE: M. Kale [drmkalex@yahoo.com - FAX: +90 (248) 2132001]. Department of Virology, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy. 15030 Burdur, Turkey.

INTRODUCTION

Respiratory tract problems in cattle settled in plants can be caused by many pathogens [19]. Especially viral pathogens are the most commonly seen factors [20]. BRSV has an important place among pneumonic cases seen in cattle. It is frequently seen especially in calves and calves younger than 1 year. In calves younger than six months, it causes acute interstitial pneumonia proceeding with alveolitis and bronchiolitis [26].

Pelargonium sidoides (PS) is a kind of plant used traditionally by native people in coastal areas of South Africa. The extract from the root of PS is a medicine whose effectiveness has been proven by various fitomedical studies (*in vitro*, *in vivo* and clinical). PS contains phenolic and cinnamic acids, tannins, flavonoids and coumarins. Towards the end of the 20th century, an effective and reliable herbal medicine called Umckaloabo was developed to cure acute bronchitis especially in children and to cure infections of upper respiratory tract obstructions [11]. Umckaloabo (EPs®7630) is an ethanolic root extract of PS roots [4] and its antibacterial, antiviral and immunomodulator features have been revealed by many studies and its therapeutic effects were determined.

This medicine is generally used for upper respiratory tract infections in human medicine [2,6]. The medicine was proved to be effective for microorganisms and immune systems [10,13], by every experimental, observational and clinical study performed with PS and its extracts. However, there is no study on its usage in veterinary medicine. In this regard, deeper knowledge and research are needed on the effectiveness of PS and its extracts for diseases both in veterinary and human medicine.

MATERIALS AND METHODS

Animals and properties of the management

In this study, we worked between 2015 and 2016 years in a stock cattle farming plant located in central Burdur Kışla district in Turkey. In this management, we worked on 40 Holstein calves aged 6-8 months old (weighing between 120-200 kg) showing respiratory system symptoms (cough, 40-41°C fever, inappetency, serious nasal flow, drying in nasolabial area, increase in respiratory rate, foamy nasal flow and dyspnea) depending on acute and peracute proceeding pneumonia. Symptoms of animals were determined by plant veterinary surgeon by getting help. The plant the study was applied was semi-outdoor with linoleum ceiling, brick side walls and soil ground. The animals were always in contact in three different sections (120 kg, 150 kg, 180-200 kg) seperated by iron paddocks. There was a feedbox and a waterbowl in each section. Within the plant, adult stock cattle were located in another area 200 meters away. During the study, no practice of vaccination or antibiotics was applied on testing and control group animals used in the study. The fact that the animals had no vaccination for bovine herpesvirus type 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine parainfluenza virus type 3 (BPIV-3), bovine respiratory syncytial virus (BRSV) and bovine adenovirus type 3 (BAV-3) before the study was confirmed by getting information from plant veterinary surgeon.

Blood sampling of animals to be used in the study

In the study, blood samples taken from *v. jugularis* of calves showing respiratory system symptoms (those under treatment and control groups) were collected in 10 mL sterile vacuum tubes[1] containing no chemical substances. Blood samplings of BRSV seropositive animals (testing and control groups) were taken on days 1st, 3rd, 5th, 7th, 10th and 14th. Samples were taken to lab with cold chain and centrifuged at 720 *g* in due form. The obtained serum samples were taken into eppendorf tubes[2] and tested within the same day. Besides, blood samples of the same animals were collected in 8 mL EDTA sterile vacuum tubes[1] on day 14th in order to detect whether there were changes in haematological parameters.

Applying Umckaloabo/ EPs®7630 liquid extract on animals to be used in the study

In the study, Umckaloabo/ EPs®7630 liquid extract Umca solution[3] was used. The compound of this commercial liquid extract includes 80 g of PS root liquid extract as the active substance per 100 g of solution and ethanol and glycerol as dissolver and protector. Commercial introduction of Umca solution includes 20 and 50 mL self-dropping glass bottles.

In our study, the usage of Umca solution in calves was determined by considering the average adult human weight. Around the world, the average adult human weight was found as 62.0 kg (condensed between 58.8 and 74.6) [27] and this figure was ac-

cepted as 60 kg. Accordingly, we applied 60 drops of Umca solution for calves weighing 120 kg, 75 drops for those weighing 150 kg and 90 drops for those weighing between 180-200 kg. Human beings are advised to consume Umca solution by dropping it into some amount of water. In the study, standard amount (100 mL) of sterilized distilled water was used.

In the study, for calves showing respiratory system symptoms and found BRSV seropositive in their blood serum samples, morning, noon and night between days 1st-14th, Umckaloabo/ EPs®7630 commercial liquid extract was homogenized in plastic containers and applied through oral route by injectors as 60 drops in 100 mL water for calves weighing 120 kg (Testing 1, Testing 2, Testing 3), 75 drops in 100 mL water for those weighing 150 kg (Testing 4, Testing 5, Testing 6) and 90 drops in 100 mL water for those weighing between 180-200 kg (Testing 7, Testing 8, Testing 9). No treatment application was performed for animals in control group (Control 1-10).

ELISA (blood)-Serological diagnostic method of respiratory tract infections

Respiratory ELISA kit[4] commercial testing product was used in order to detect antibody presence in the plant against BHV-1, BVDV, BRSV, BPIV-3 and BAV-3 in blood serum samples taken on day 1st from 40 stock calves showing respiratory system symptoms. The application was performed according to kit procedure.

ELISA (blood)-Calculating antibody titers with BRSV IgG

Twenty BRSV seropositive calves were classified as 10 for control group and 10 for testing group using Respiratory ELISA kit[4] commercial testing product. In blood samples of both groups taken on days 1st, 3rd, 5th, 7th, 10th and 14th, BRSV titers were detected using BRSV IgG Antibody Test Kit[5]. Blood samples were diluted with sterile saline solution at a rate of 1/2 according to $\log_2$ base. Except for negative and positive controls, dilutions for each sample were performed within 8 compartments of the plate at rates of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256. Other stages of the application were carried out according to kit procedure.

Determining haematological parameters

Blood samples taken into K3EDTA tubes on day 14th from BRSV seropositive calves classified as 10 for testing and 10 for control groups were examined by full blood device[6] for haematological studies.

Statistical analysis

In this study, 2 sample t-test analysis was performed to compare haematological parameters between testing and control groups. The analysis was carried out using Minitab[7] programme. Values smaller than $P < 0.01$ or $P < 0.05$ were statistically considered important.

RESULTS

Respiratory system symptoms of animals

In the study, respiratory system symptoms in 10 Holstein calves within the control group continued and subsided slightly. Respiratory system symptoms in 10 Holstein calves within the testing group subsided and got better. Respiratory system symptoms of animals were determined by the help of plant veterinary surgeon.

ELISA (blood) - Serological diagnostic results of respiratory tract infections

Antibody presence in the plant against BHV-1, BVDV, BRSV, BPIV-3 and BAV-3 in blood serum samples taken on day 0. from 40 stock calves showing respiratory system symptoms was examined. All samples were found as BRSV seropositive while other factors were found seronegative in terms of BHV-1, BVDV, BPIV-3 and BAV-3.

ELISA (blood)-Calculating antibody titers with BRSV IgG in testing and control groups

Twenty BRSV seropositive calves were classified as 10 for testing and 10 for control groups. BRSV titers were detected in blood samples taken from both groups on days 1st, 3rd, 5th, 7th, 10th and 14th (Tables 1 and 2). Blood samples were diluted at a rate of ½ according to $\log_2$ base. Except for negative and positive controls, dilutions for each sample were performed within 8 compartments of the plate at rates of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256. At the end of day 14th, in 9 out of 10 animals in testing group (90%) that were given Umckaloabo/ EPs®7630 liquid extract, BRSV antibody titer increased and no change was observed in one of them (10%). In 6 out of 10 animals (60%) in control group, BRSV antibody titer increased, no change was observed in three of them (30%) and it decreased in one of them (10%). In testing group, BRSV antibody titer started to increase in 6 out of 10 animals (60%) on day 3rd, in 1 of them (10%) on day 5th, in 1 of them (10%)

on day 10th and in one of them (10%) on day 14th. In control group, BRSV antibody titer increased in 3 out of 10 animals (30%) on day 3rd. and in 3 of them (30%) on day 5th (Tables 1 and 2).

Haematological parameter results

When haematological values (WBC, LYM, MID, GRA, LY%, MI%, GR%, RBC, HGB, HCT, MCV, MCH, MCHC, PLT) in blood samples taken on day 14th from animals in testing and control groups were comparatively studied, no statistical importance ($P < 0.01$ or $P < 0.05$) or difference was detected between both groups (Tables 3 and 4) (Table 5). In brief, the possibility of averages between both groups to be different from each other was found higher than 1% and 5%.

Table 1. BRSV antibody titer results in the testing group.

Time (Days)	Testing-1	Testing-2	Testing-3	Testing-4	Testing-5	Testing-6	Testing-7	Testing-8	Testing-9	Testing-10
1st	1/8	1/256	1/32	1/128	1/64	1/128	1/64	1/64	1/128	1/32
3rd	1/16	1/256	1/16	1/256	1/128	1/256	1/64	1/64	1/256	1/64
5th	1/16	1/256	1/32	1/256	1/256	1/256	1/64	1/128	1/256	1/128
7th	1/64	1/256	1/32	1/256	1/256	1/256	1/64	1/128	1/256	1/128
10th	1/128	1/256	1/64	1/256	1/256	1/256	1/64	1/128	1/256	1/128
14th	1/256	1/256	1/128	1/256	1/256	1/256	1/128	1/128	1/256	1/256

Table 2. BRSV antibody titer results in the control group.

Time (Days)	Control-1	Control-2	Control-3	Control-4	Control-5	Control-6	Control-7	Control-8	Control-9	Control-10
1st	1/64	1/32	1/32	1/256	1/16	1/4	1/64	1/128	1/4	1/8
3rd	1/64	1/64	1/32	1/256	1/32	1/4	1/64	1/128	1/8	1/8
5th	1/256	1/256	1/128	1/256	1/256	1/32	1/256	1/256	1/64	1/128
7th	1/128	1/128	1/128	1/256	1/256	1/32	1/128	1/128	1/64	1/128
10th	1/128	1/128	1/128	1/256	1/256	1/32	1/128	1/128	1/32	1/128
14th	1/64	1/64	1/64	1/128	1/64	1/32	1/64	1/128	1/32	1/16

Table 3. Haematological values of the testing groups (Day 14th).

No	WBC 4-12x10^9 cell/µL	LYM 2.5-7.5x10^9 cell/µL	MID 0-0.84x10^9 cell/µL	GRA 0.6-6.7x10^9 cell/µL	LY% 45-75%	MI% 2-7%	GR% 15-65%	RBC 5-10x10^{12}/L	HGB 8-15 g/dL	HCT 24-46%	MCV 40-60 µ	MCH 11-17 pg	MCHC 30-36 g/dL	PLT 100-800x 10^9/L
T-1	10.09	3.79	0.10	6.21	37.5	1.0	61.5	9.08	8.4	26.28	29	9.2	31.9	629
T-2	11.46	3.48	1.01	6.97	30.4	8.8	60.8	8.13	8.0	24.90	31	9.8	32.1	590
T-3	9.91	6.08	0.48	3.35	61.4	4.8	33.8	9.67	9.4	29.34	30	9.7	31.9	796
T-4	8.52	3.35	0.08	5.09	39.3	1.0	59.7	8.12	8.8	28.66	35	10.9	30.8	691
T-5	10.75	4.80	0.09	5.86	44.7	0.8	54.5	9.83	9.7	30.06	31	9.8	32.2	676
T-6	7.80	4.57	0.57	2.65	58.7	7.4	34	9.53	9.8	29.55	31	10.3	33.2	693
T-7	11.28	7.44	0.11	3.73	66.0	1.0	33.1	8.20	8.6	28.08	34	10.5	30.6	330
T-8	10.11	3.63	0.08	6.40	35.9	0.8	63.3	7.83	7.8	24.56	31	9.9	31.7	475
T-9	9.37	6.60	0.53	2.24	70.5	5.6	23.9	6.62	7.7	22.96	35	11.6	33.5	213
T-10	9.73	4.05	0.22	5.46	41.6	2.3	56.1	8.69	8.1	24.41	28	9.3	33.2	689

T: Testing; WBC: Leucocyte; LYM: Lymphocyte; MID: Monocyte; GRA: Granulocyte; LY%: Lymphocyte%; MI%: Monocyte%; GR%: Granulocyte%; RBC: Erythrocyte; HGB: Hemoglobin; HCT: Hemotocrit; MCV: Average cell volume; MCH: Average cell hemoglobin; MCHC: Average cell hemoglobin concentration; PLT: Platelet.

Table 4. Haematological values of the control groups (Day 14th).

No	WBC 4-12x 10^9 cell/µL	LYM 2.5-7.5x 10^9 cell/µL	MID 0-0.84x10^9 cell/µL	GRA 0.6-6.7x10^9 cell/µL	LY% 45-75%	MI% 2-7%	GR% 15-65%	RBC 5-10x 10^{12}/L	HGB 8-15 g/dL	HCT %24-46	MCV 40-60 µ	MCH 11-17 pg	MCHC 30-36 g/dL	PLT 100-800x 10^9/L
C-1	14.72	6.73	0.13	7.86	45.7	0.9	53.4	10.18	9.7	29.45	29	9.5	32.8	748
C-2	4.80	0.87	0.06	3.88	18.1	1.1	80.8	7.69	8.5	26.73	35	11.0	31.7	730
C-3	14.22	4.99	1.34	7.89	35.1	9.5	55.4	8.72	8.0	25.16	29	9.2	31.8	820
C-4	7.27	1.94	0.18	5.15	26.7	2.5	70.8	9.02	8.4	25.69	28	9.4	32.9	505
C-5	9.27	2.18	0.82	6.26	23.6	8.9	67.6	8.98	8.7	26.45	29	9.7	32.8	588
C-6	8.30	2.40	0.72	5.17	29.0	8.7	62.3	8.18	7.7	24.33	30	9.4	31.6	793
C-7	11.71	6.90	0.42	4.39	58.9	3.6	37.5	9.77	9.2	27.83	28	9.4	32.9	803
C-8	8.97	3.79	0.78	4.40	42.2	8.7	49.1	9.34	8.6	25.32	27	9.3	34.1	351
C-9	9.60	4.47	0.13	5.00	46.6	1.3	52.1	8.64	8.2	23.97	28	9.5	34.1	728
C-10	13.03	7.51	0.72	4.80	57.7	5.5	36.8	9.00	9.3	28.08	31	10.4	33.3	504

C: Control; WBC: Leucocyte; LYM: Lymphocyte; MID: Monocyte; GRA: Granulocyte; LY%: Lymphocyte%; MI%: Monocyte%; GR%: Granulocyte%; RBC: Erythrocyte; HGB: Hemoglobin; HCT: Hemotocrit; MCV: Average cell volume; MCH: Average cell hemoglobin; MCHC: Average cell hemoglobin concentration; PLT: Platelet.

Table 5. Comparing the average haematological values of the testing and the control groups statistically (Day 14th).

No Mean ± SD	WBC $4\text{-}12x10^9$ cell/µL	LYM 2.5-$7.5x10^9$ cell/µL	MID $0\text{-}0.84x10^9$ cell/µL	GRA $0.6\text{-}6.7x10^9$ cell/µL	LY% 45-75%	MI% 2-7%	GR% 15-65%	RBC $5\text{-}10x10^{12}$/L	HGB 8-15 g/dL	HCT %24-46	MCV 40-60 µ	MCH 11-17 pg	MCHC 30-36 g/dL	PLT 100-800x 10^9/L
Testing	9.90 ± 1.14	4.78 ± 1.44	0.327 ± 0.31	4.80 ± 1.68	48.6 ± 14.2	3.35 ± 3.05	48.1 ± 15.0	8.57 ± 0.99	8.63 ± 0.78	26.88 ± 2.56	31.50 ± 2.42	10.10 ± 0.74	32.11 ± 0.98	578 ± 184
Control	10.19 ± 3.18	4.18 ± 2.33	0.530 ± 0.41	5.48 ± 1.41	38.4 ± 14.1	5.07 ± 3.60	56.6 ± 14.1	8.95 ± 0.72	8.63 ± 0.62	26.30 ± 1.75	29.40 ± 2.27	9.68 ± 0.57	32.80 ± 0.90	657 ± 160
P	*P = 0.40* NS*	*P* = 0.75 NS*	*P* = 0.12 NS*	*P* = 0.17 NS*	*P* = 0.94 NS*	*P* = 0.13 NS*	*P* = 0.10 NS*	*P* = 0.17 NS*	*P* = 0.50 NS*	*P* = 0.72 NS*	*P* = 0.97 NS*	*P* = 0.91 NS*	*P* = 0.059 NS*	*P* =0 .16 NS*

P: Predictive value; Mean±SD: Mean± Standart deviation; *: $P < 0.01$ or $P < 0.05$; WBC: Leucocyte; LYM: Lymphocyte; MID: Monocyte; GRA: Granulocyte; LY%: Lymphocyte%; MI%: Monocyte%; GR%: Granulocyte%; RBC: Erythrocyte; HGB: Hemoglobin; HCT: Hemotocrit; MCV: Average cell volume; MCH: Average cell hemoglobin; MCHC: Average cell hemoglobin concentration; PLT: Platelet.

DISCUSSION

BRSV played the biggest role in respiratory system epidemics in calves in 20 herds located in Middle and Southeast France [24]. Similarly, BRSV and BPIV-3 factors were regarded responsible for respiratory infection epidemics in calves in Ireland [5]. Within naturally developed infections in BRSV endemic areas, the infection mostly develop in calves smaller than 6 months old and adult animals become immune. However, together with virus introduction into areas where BRSV non-endemic and BRSV seronegative adult animals are located, animals of all ages might become infected [8,25].

On our day, when vaccinations against RSV infections are not available, some treatment applications that have moderate or limited efficiency such as palivizumab (monoclonal antibody against RSV fusion protein) and ribavirin (nucleoside analogue) can be performed. That's why developing a new antiviral medicine against RSV infections is needed. In this regard, some herbal origin natural products as an anti-RSV activity have been developed. Among these herbs, especially tannin substances incativate RSV particles and prevent the virus from binding and entering into the cell including fusion stages. Therefore, respiratory system symptoms resulting from RSV, including inflammations in respiratory tracts, can be cured by some natural herbal products [12]. Applications of herbal origin medicine on many respiratory system infections to this day have been performed on humans and experimental animals. About this topic, no study is available on calves and cattle. That's why our results from the study were mostly evaluated and compared with data obtained from people.

In our study, at the end of day 14th, in 9 out of 10 animals in testing group (90%) that were given Umckaloabo/ EPs®7630 liquid extract, BRSV antibody titer increased and no change was observed in one of them (10%). In 6 out of 10 animals (60%) in control group, BRSV antibody titer increased, no change was observed in three of them (30%) and it decreased in one of them (10%). In testing group, BRSV antibody titer started to increase in 6 out of 10 animals (60%) on day 3rd, in 1 of them (10%) on day 5th, in 1 of them (10%) on day 10th and in one of them (10%) on day 14th. In control group, BRSV antibody titer increased in 3 out of 10 animals (30%) on day 3rd and in 3 of them (30%) on day 5th. In case of naturally developed

BRSV infection in calves aged 6-7 months old, IgM and IgG antibody titers increased. In case of natural infections based on the respiratory system developing during acute period especially in calves, IgM and IgG respond improved [28]. PS extracts were confirmed by some testing to have displayed decent activations towards influenza viruses (H1N1 and H3N2), coxsackie A9 virus, human coronavirus, RSV, PI-3, HSV-1 and 2 [15,17,22]. These two research groups [15,22] emphasized that EPs®7630 extract was more active for membranous viruses rather than non-membranous ones. Michaelis *et al*. [15] searched the effectiveness of EPs®7630 extract in replication of respiratory viruses. In the study, EPs®7630 extract was practised on adenovirus 3 and 7, RSV, human rhinovirus 16, H1N1 and H3N2 influenza, coronavirus (HCO-229E) and coxsackie virus A9. EPs®7630 was determined to have interfered with cytopathogenic effect (CPE) caused by RSV, coronavirus, H1N1 and H3N2 influenza, parainfluenza type 3 (PI-3) and coxsackie virus A9. Besides, effects of EPs®7630 on H1N1 and H3N2 influenza, RSV, coronavirus (HCO-229E), PI-3 and coxsackie virus A9 titers were studied and EPs®7630 was found to have caused a decrease in all sensitive virus titers depending on the dosage. In our study, after day 14th, antibody titer of the testing group given Umckaloabo/EPs®7630 liquid extract was found higher than the control group given no extract. In addition, on day 3rd, antibody increase was most detected in animals in testing group.

Gezer and Turan [9] studied the effects of adding geranium root (PS) extract to fishmeal at varying rates on growth of small carps, body composition and blood parameters. At the end of testing, compared to control group, in the group containing 5 mL 100 g^{-1} geranium root, a statistically significant difference was detected in leucocyte, hemoglobin and erythrocyte hemoglobin concentration levels ($P < 0.01$). However, as a result of the statistical analysis performed at the end of testing, no difference was found between control group and groups given geranium root extract in levels of erythrocyte, hematocrit, erythrocyte volume and erythrocyte hemoglobin ($P > 0.05$).

Tunc and Patıroglu [23] evaluated the effectiveness of PS extract which had both antiviral and immunomodulator efficiency for better symptoms during upper respiratory tract infection of patients followed up due to temporary hypogammaglobulinemia of sucking babies. During pre and post treatment periods, no statistical difference was found between placebo group and the group given PS in terms of blood parameters [leucocyte, hemoglobin, thrombocyte, prothrombin time] ($P < 0.05$).

Matthys and Heger [14] stated that, for patients believed to have an infectious disease, an increase in erythrocyte sedimentation rate appeared in patients using EPs®7630 liquid extract (10 out of 108 patients), placebo group patients (10 out of 109) and changes in leucocyte count appeared in patients using EPs®7630 liquid extract (4 out of 108 patients), placebo group patients (5 out of 109). In this study, when the haematological values (leucocyte, lymphocyte, monocyte, granulocyte, lymphocyte%, monocyte%, granulocyte%, erythrocyte, hemoglobin, hematocrit, average cell volume, average cell hemoglobin, average cell hemoglobin concentration, platelet) in blood samples taken on day 14th (post treatment) from animals in testing and control groups were comparatively examined, no statistical importance ($P < 0.01$ or $P < 0.05$) and difference was found between both groups.

An acute cough is usually seen in viral infections (common cold) in respiratory systems. Sore throat, red nose, headache, body pain, fatigue and fever might also exist. Coughing reflections start due to damage on respiratory tract epithelium nerve sensors or oscillation of inflammatory mediators. When Agbabiaka *et al*. [1] examined 5 studies performed in various years, they performed PS extracts as standard treatment for patients with acute bronchitis. They also included placebo group in the study. They compared a group on which PS extract was applied with another group on which antibiotics and acetylcysteine was not applied and 5 groups on which PS extract was applied with another group on which placebo was applied. In the study, they found that PS was effective in the group with acute bronchitis and acute bronchitis symptoms decreased significantly in these patients on day 7th.

Tahan and Yaman [21] applied PS extract (EPs®7630) for 5 days on 61 children with asthma progressing due to upper respiratory tract viral infections. In the study, fever and muscle pains in the group with PS treatment were statistically ($P > 0.05$) not found more important than in the group with no treatment and coughing frequency, nasal congestion and asthma attack cases in the group with PS treatment were statistically ($P > 0.05$) found more important

than in the group with no treatment. They stated that asthma attacks in the group with PS treatment were seen less frequently.

Viruses, as the primary factor, are usually considered to be responsible for appearing respiratory tract infections and trigger asthma attacks. Increasing respiratory tract inflammations were the sudden reason of asthma attacks and respiratory tract inflammations increasing during viral infections. As a result of decreasing the duration of viral infection efficiency, development of respiratory tract inflammations could be decreased and asthma attacks could be discarded [21]. Bao *et al*. [3] searched antitussive, secretolytic and anti-inflammatory effects of EPs®7630 in experimental animals. After the study, the coughing frequency of experimental animals with cough decreased significantly depending on the dosage and cough delay period became longer. Secretolytic activity similarly decreased in rats. Lesions in lungs, trachea and bronch tissues caused by acute bacterial bronchitis depending on EPs®7630 in rats regressed histopathologically. As a result, researchers were of the opinion that using EPs®7630 therapeutically in respiratory system infections was effective. For 7 days, Umca solution was applied as 3x30 drop/day for patients over 18 years of age and showing acute bronchitis symptoms. Especially bronchitis severity score (BSS) [cough, mucus, dyspnea, chest pain while coughing, fever over 38.5°C] was determined for patients. At the end of the study, on day 7, BSS score decreased and individual symptoms decreased more distinctly than placebo group. On the fourth day of treatment applications, 69% of patients and 33% of placebo group showed improvement [18].

Viruses are the most commonly seen factors in most of acute respiratory tract infections. Acute coughing usually accompanies respiratory tract viral infections. Moreover, other symptoms such as sore throat, nasal flow, headache, joint pains, fatigue and fever can be seen [3]. In our study, in 10 Holstein calves in the control group, respiratory system symptoms (cough, fever, inappetency, serous nasal flow, drying on nasolabial area, increase in respiration rate, foamy nasal flow and dyspnea) continued and decreased slightly. In 10 Holstein calves in the testing group, respiratory system symptoms decreased and got better.

PS not only has an antiviral efficiency but also can increase immune response through secretory Ig A, IL-15, IL-6, IFN-ɣ ve TNF-α and shorten disease period [13]. Effects of PS on mucociliary system is believed to play a role in decreasing coughing frequency. The relation between EPs®7630 and cilia pulse frequency was searched in human cilia nose epithelial cell cultures. At the end of the study, EPs®7630 was thought to have increased cilia pulse frequency significantly depending on the dosage [16]. In conclusion, PS is thought to be effective through many mechanisms such as cilia functions, immunoglobulins, cytokines and chemokines, phagocytic functions, intracell killing mechanisms and antiviral efficieny, to shorten viral upper respiratory tract infection period and to supress attack development by preventing inflammatory cases from progressing [7,22].

In this study, by using ELISA (IgG), antibody presence against BRSV was detected in Holstein calves aged 6 months and over showing symptoms of respiratory problems. Out of 20 BRSV seropositive calves, 10 of them were classified as control group and the other ten as testing group. BRSV antibody titers were also detected in blood samples of both groups on day 1st Umckaloabo/EPs®7630 liquid extract was given through oral route to animals in testing group according to their weights for 14 days morning, noon and night. No application was performed on animals in control group. BRSV antibody titers were detected in blood samples of animals in both groups taken on days 1st, 3rd, 5th, 7th, 10th and 14th.

In BRSV seropositive stock calves showing respiratory system symptoms, applying Umckaloabo/EPs®7630 liquid extract helped antibody titer to increase and symptoms (cough, fever, inappetency, serous nasal flow, drying on nasolabial area, increase in respiration rate, foamy nasal flow and dyspnea) decreased. The increase in antibody titers started from day 3th especially after applying Umckaloabo/EPs®7630 liquid extract. Besides, when haematological values in blood samples taken on day 14th from animals in testing and control groups were comparatively studied, no statistical difference was detected between both groups. During clinical observations, no side effect was observed either in testing group on which extract was applied.

MANUFACTURERS

[1]Vacutest. Arzergrande, Italy.

[2]Labor-Teknik. Istanbul, Turkey.

[3]Abdi İbrahim İlaç Pazarlama A.Ş. İstanbul, Turkey.

[4]BioX Diagnostics, Pentakit BIO K 028, Belgium.

[5]IDEXX Laboratories. Saint Denis, France.

[6]Abacus Junior Vet Hematology Analyzer. Wien, Austria.

[7]Minitab15 (version15). State College, PA, USA.

***Acknowledgements*.** This study has been supported by Mehmet Akif Ersoy University Coordinatorship of Scientific Research Projects with the project number 0246-YL-14.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Agbabiaka T.B., Guo R. & Ernst E. 2008.** *Pelargonium sidoides* for acute bronchitis: a systematic review and meta-analysis. *Phytomedicine*. 15(5): 378-385.

2 **Balbani A.P.S., Montovani J.C. & Carvalho L.R. 2009.** Pharyngotonsillitis in children: view from a sample of pediatricians and otorhinolaryngologists. *Brazilian Journal of Otorhinolaryngology*. 75(1): 139-146.

3 **Bao Y., Gao Y., Koch E., Pana X., Jina Y. & Cui X. 2015.** Evaluation of pharmacodynamic activities of EPsR 7630, a special extract from roots of *Pelargonium sidoides*, in animals models of cough, secretolytic activity and acute bronchitis. *Phytomedicine*. 22(4): 504-509.

4 **Brendler T. 2009.** Umckaloabo: From a Patent Remedy to a Modern Herbal Pharmaceutical Based on *Pelargonium sidoides* with Clinically Proven Efficacy. In: Juliani R.H., Simon J.H. & Ho C.T. (Eds). *African Natural Plant Products: New discoveries and Challenges in Chemistry and Quality*. Oxford: Oxford University Press, pp.295-319.

5 **Bryson D.G., McFerran J.B., Ball H.J. & Neill S.D. 1978.** Observations on outbreaks of respiratory disease in housed calves. Epidemiological and clinical findings. *The Veterinary Record*. 103(22): 485-489.

6 **Chuchalin A.G., Berman B. & Lehmacher W. 2005.** Treatment of acute bronchitis in adults with a *Pelargonium sidoides* preparation (EPs® 7630): A randomized, double-blind, placebo-controlled trial. *Explore (NY)*. 1(6): 437-445.

7 **Conrad A., Hansmann C., Engels I., Daschner F.D. & Frank U. 2007.** Extract of *Pelargonium sidoides* (EPs® 7630) improves phagocytosis, oxidative burst, and intracellular killing of human peripheral blood phagocytes *in vitro*. *Phytomedicine*. 14(6): 46-51.

8 **Elvander M. 1996.** Severe respiratory disease in dairy cows caused by infection with bovine respiratory syncytial virus. *The Veterinary Record*. 138(5): 101-105.

9 **Gezer A. & Turan F. 2009.** Sardunya kökü (*Pelargonium sidoides*) ekstraktının yavru sazan (*Cyprinus carpio* l. 1758)'larda büyüme, vücut kompozisyonu ve kan parametreleri üzerine etkileri. 54syf. Hatay, Yüksek lisans tezi (Su Ürünleri Fakültesi)-Su Ürünleri Anabilim Dalı Programı, Mustafa Kemal Üniversitesi.

10 **Helfer M., Koppensteiner H., Schneider M., Rebensburg S., Forcisi S., Muller C., Schmitt-Kopplin P., Schindler M. & Brack-Werner R. 2014.** The root extract of the medicinal plant *Pelargonium sidoides* is a potent HIV-1 attachment inhibitor. *PLOS ONE*. 9(1): e.87487- 87487.

11 **Kolodziej H. 2007.** Fascinating metabolic pools of *Pelargonium sidoides* and *Pelargonium reniforme*, traditional and phytomedicinal sources of the herbal medicine Umckaloabo. *Phytomedicine*. 14(6): 9-17.

12 **Lin L.T., Hsu W.C. & Lin C.C. 2014.** Antiviral natural products and herbal medicines. *Journal of Traditional and Complementary Medicine*. 4(1): 24-35.

13 **Luna L.A., Bachib A.L.L., Novaes e Brito R.R., Eid R.G., Suguri V.M., Oliveira P.W., Gregorio L.C. & Vaisberg M. 2011.** Immune responses induced by *Pelargonium sidoides* extract in serum and nasal mucosa of athletes after exhaustive exercise: modulation of secretory IgA, IL-6 and IL-15. *Phytomedicine*. 18(4): 303-308.

14 **Matthys H. & Heger M. 2007.** Treatment of acute bronchitis with a liquid herbal drug preparation from *Pelargonium sidoides* (EPs® 7630): a randomised, double-blind, placebo-controlled, multicentre study. *Current Medical Research Opinion*. 23(2): 323-331.

15 **Michaelis M., Doerr H.W. & Cinatl J. 2011.** Investigation of the influence of EPs 7630, a herbal drug preparation from Pelargonium sidoides, on replication of a broad panel of respiratory viruses. *Phytomedicine*. 18(5): 384-386.

16 **Neugebauer P., Mickenhagen A., Siefer O. & Walger M. 2005.** A new approach to pharmacological effects of ciliary beat frequency in cell cultures-exemplary measurements under *Pelargonium sidoides* extract (EPs 7630). *Phytomedicine*. 12(1-2): 46-51.

17 **Schnitzler P., Schneider S., Stintzing F.C., Carle R. & Reichling J. 2008.** Efficacy of an aqueous *Pelargonium sidoides* extract against herpesvirus. *Phytomedicine*. 15(12): 1108-1116.

18 **Schulz V. 2007.** Liquid herbal drug preparation from the root of *Pelargonium sidoides* is effective against acute bronchitis: Results of a double-blind study with 124 patients. *Phytomedicine*. 14(6): 74-75.

19 **Sharp M.W., Williams R.L. & Munro R. 1993.** Acute respiratory distress syndrome in yearling cattle. *The Veterinary Record*. 132(18): 467-468.

20 **Stott E.J., Thomas L.H., Collins A.P., Crough S., Jebbet J., Smıth G.S., Luther P.D. & Caswell R. 1980.** A survey of virus infections of the respiratory tract of cattle and their association with disease. *The Journal of Hygiene*. 85(2): 257-270.

21 **Tahan F. & Yaman M. 2013.** Can the *Pelargonium sidoides* root extract EPs 7630 prevent asthma attacks during viral infections of the upper respiratory tractin children? *Phytomedicine*. 20(2): 148-150.

22 **Theisen L.L. & Muller C.P. 2012.** EPs 7630 (umckaloabo), an extract from *Pelargonium sidoides* roots, exerts anti-influenza virus activity *in vitro* and *in vivo*. *Antiviral Research*. 94(2): 147-156.

23 **Tunc A. & Patıroglu T. 2010.** Süt çocuğunun geçici hipogammaglobulinemisi olan hastalarda üst solunum yolu enfeksiyonu tedavisinde *Pelargonium sidoides* etkinliğinin değerlendirilmesi. 67syf. Kayseri, Yüksek lisans tezi (Tıp Fakültesi)-Çocuk Sağlığı ve Hastalıkları Anabilim Dalı Programı, Erciyes Üniversitesi.

24 **Valarcher J.F. & Hagglund S. 2006.** Viral respiratory infections in cattle. In: *XXIV. World Buiatrics Congress* (Nice, France). Available at:< http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.500.5102&rep=rep1&type=pdf>. [Accessed online in September 2016].

25 **Poel van der W.H.M., Kramps J.A., Middel W.G.J., Oirschot van J.T. & Brand A. 1993.** Dynamics of bovine respiratory syncytial virus: A longitudinal epidemiological study in dairy herds. *Archives of Virology*. 133(3-4): 309-321.

26 **Poel van der W.H.M., Brand A., Kramps J.A. & Oirschot van J.T. 1994.** Respiratory syncytial virs infections in human beings and in cattle, an epidemiological review. *Journal of Infection*. 29(2): 215-228.

27 **Walpole S.C., Prieto-Merino D., Edwards P., Cleland J., Stevens G. & Roberts I. 2012.** The weight of nations: an estimation of adult human biomass. *BMC Public Health*. 12(439): 1-6.

28 **Westenbrink F. & Kimman T.G. 1987.** Immunoglobulin M-specific enzyme-linked immunosorbent assay for serodia gnosis of bovine respiratory syncytial virus infections. *American Journal of Veterinary Reserach*. 48(7): 1132-1137.