An evaluation of the impact of aloe vera and licorice extracts on the course of experimental pigeon paramyxovirus type 1 infection in pigeons

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ABSTRACT The progressive decrease in the efficiency of synthetic drugs has prompted research into phytogenic feed additives with potentially immunomodulatory and anti-infective properties. Complex diseases with a mixed etiology, including viral, pose a growing problem in domestic pigeons. The aim of this study was to determine the effectiveness of various doses of aloe vera and licorice extracts on the course of experimental PPMV-1 infection in pigeons. The experiment was performed on pigeons divided into 5 groups, including one control group and 4 experimental groups, which were orally administered aloe vera or licorice extracts at 300 or 500 mg/kg BW for 7 d after experimental inoculation with PPMV-1. On d 4, 7, and 14 after inoculation, cloacal swabs and samples of organs were collected from 4 birds in each group. The samples were analyzed to determine the copy number of PPMV-1 RNA by TaqMan qPCR. The results indicate that licorice and aloe vera extracts inhibited PPMV-1 replication by decreasing viral RNA copy numbers in the examined organs. The most inhibitory effect was observed in pigeons receiving aloe vera extract at 300 mg/kg BW, for which PPMV-1 RNA copy numbers were approximately 7-fold lower (brain), 9-fold lower (kidneys), and 14-fold lower (liver) than in the control group. The results of this study point to the potentially antiviral effects of aloe vera and licorice extracts in pigeons infected with PPMV-1. To the best of our knowledge, this is the first study to investigate the antiviral properties of aloe vera and licorice extracts in domestic pigeons.

Key words: aloe vera, licorice, pigeon, PPMV-1, TaqMan qPCR

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INTRODUCTION

Medicinal plants have been used for millennia for the prevention and treatment of diseases. Traditional medicine enjoys widespread attention (Hoareau and DaSilva, 1999). The popularity of herbal treatments decreased considerably when synthetic drugs were introduced for use. However, the overuse of medications has gradually increased drug resistance in pathogenic microorganisms. In recent years, the dwindling efficacy and rising production cost of synthetic drugs have revived researchers' interest in medicinal plants. At present, around 25% of popular drugs contain plant ingredients (Abd-Alla et al., 2012).

Aloe vera (*Aloe barbadensis Miller*) has been used as a medicinal plant for centuries, and it has been relatively well researched. Recent studies have considerably expanded our knowledge about the medicinal properties and applications of aloe vera. The succulent plants of the genus *Aloe* belong to the lily family (*Liliaceae*) (Choi and Chung, 2003). Aloe vera is cultivated for its fleshy leaves that contain mainly latex and gel. Aloe vera gel contains 98.5 to 99.5% water and 75 biologically active compounds (Darabighane and Nahashon, 2014). Polysaccharides account for around 60% of the solid fraction of aloe vera gel. The most important polysaccharide is acemannan, which is one of the most potent immunomodulators of plant origin (Darabighane et al., 2012). Aloe vera contains anthraquinones, saccharides, vitamins, enzymes, minerals, and hormones that are responsible for its anti-inflammatory, antibacterial, antifungal, and anticarcinogenic properties (Surjushe et al., 2008). Aloe vera inhibits the attachment and entry of the influenza virus, cytomegalovirus, human herpes virus, and polio virus into host cells (Sydiskis et al., 1991).

Licorice (*Glycyrrhiza glabra*) is also a popular medicinal plant. This herbaceous plant belongs to the legume family (*Fabaceae*), and it has been used in traditional

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medicine for more than 4,000 years (Shebl et al., 2012). Similarly to aloe vera, licorice demonstrates a broad spectrum of activity, including anti-allergic, anti-diabetic, and anticarcinogenic (Parvaiz et al., 2014). The licorice root is a rich source of flavonoids and triterpenoid saponins. The main chemical ingredient is glycyrrhizic acid whose concentrations in licorice root extract range from 1 to 9%, subject to latitude (Fiore et al., 2008). Glycyrrhizin has anti-inflammatory and antioxidant properties. Pompei et al., (1979) demonstrated that glycyrrhizic acid inhibits the proliferation of hepatovirus A and B, influenza virus, and HIV.

Medicinal herbs such as aloe vera and licorice are widely used as feed additives in animal nutrition. In chickens, phytogenic feed additives have been found to improve body weight gains, the composition and growth of gut microbiota, and immune function (Darabighane and Nahashon, 2014). The immunomodulatory effects of aloe vera and licorice extracts have never been studied in pigeons, to the best of our knowledge. Viral infections, in particular infections caused by the pigeon circovirus (**PiCV**), pose a growing problem in pigeon pathology (Marlier and Vindevogel, 2006; Krapež et al., 2012; Stenzel et al., 2012). The prevalence of PiCV is very high in pigeon populations around the world, and the virus probably triggers the young pigeon disease syndrome. A protocol for culturing PiCV under laboratory conditions has not yet been developed (Daum et al., 2009), whereas the pigeon variant (pigeon paramyxovirus type 1, **PPMV-1**) of the Newcastle disease virus (**NDV**), which is capable of proliferating in specific pathogen-free chicken embryos and cell cultures (Alexander and Senne, 2008), has been extensively researched, and it could be used in experimental inoculation of pigeons (Dortmans et al., 2011; Stenzel et al., 2014; Śmietanka et al., 2014).

PPMV-1 is classified as a serotype 1 avian paramyxovirus (**APMV-1**), and together with 12 other serotypes of this virus, it belongs to the genus *Avulavirus*, subfamily *Paramyxovirinae*, family *Paramyxoviridae* (Aldous and Alexander, 2001; Terregino et al., 2013; Karamendin et al., 2016). The paramyxovirus genome consists of a single negative-sense strand of RNA that contains around 15,000 base pairs encoding 6 structural proteins (Marlier and Vindevogel, 2006). APMV-1 infections can produce different clinical symptoms, subject to the virulence of viral strain. The virus has been categorized into 5 pathotypes: viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic, and asymptomatic. Velogenic viruses are most virulent, and they cause significant losses in bird populations (Alexander, 2000).

To date, the efficacy of aloe vera and licorice extracts against paramyxovirus infections has been studied only in chickens based on the results of standard laboratory tests evaluating blood biochemical parameters, weight gains, and antibody levels against NDV (Waihenya et al., 2002; Ojiezeh and Eghafona, 2015; Ojiezeh and Ophori, 2015).

Newcastle disease is a notifiable disease that has to be legally reported to the authorities, and treatment of PPMV-1 infections in pigeons is not allowed. In view of the above, this study presents basic research for in vivo assessment of the antiviral activity of aloe vera and licorice extracts against PPMV-1 in experimentally infected pigeons.

MATERIALS AND METHODS

This study was carried out in strict observance of the Act of 21 January 2005 on animal experimentation and the Regulation of the Minister of Science and Information Technology of 29 July 2005 on the National Committee for Animal Experimentation. The research protocol was approved by the Local Ethics Committee on Animal Experimentation of the University of Warmia and Mazury in Olsztyn (Authorization No. 64/2014, valid until 26 November 2017). The researchers made every effort to minimize the suffering of birds.

Virus

Pigeons were infected with the pigeon paramyxovirus serotype-1 (PPMV-1/pigeon/Poland/AR3/95) obtained from the National Veterinary Research Institute in Puławy. The isolate had ^{112}R-R-Q-K-R-F^{117} sequence at the cleavage site in the fusion protein gene, and it was classified as a pathogenic strain.

Plant Extracts

Aloe vera. The aloe vera extract was obtained by freeze/spray drying of aloe leaf juice. Five grams of the extract with maximum moisture content of 8% and bulk density of 0.3 to 0.6 g/1 mL were obtained from 1,000 g of fresh aloe vera juice.

Licorice. Dry licorice extract was obtained by spray drying of an aqueous solution of licorice root, which is a registered feed additive (European Union Register of Feed Additives, group 2b: natural products – botanically defined: CAS 68916-91-6 FEMA 2629, CoE 218, pursuant to Regulation (EC) No 1831/2003). The extract contained 20% glycyrrhizic acid, and it was characterized by maximum moisture content of 3.6% and bulk density of 0.5 g/1 mL.

Aloe vera and licorice extracts were free of pathogenic bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, in 10 g of the product.

Pigeons. Sixty 8-week-old fantail pigeons were obtained from a private breeder. The flock in the breeding facility had not been vaccinated against PPMV-1 since 2008, and it was free of the infection. Before the experiment, cloacal swabs and blood samples were collected from all birds to rule out PPMV-1 infection with the use of the method described below, and to determine the presence of antibodies against PPMV-1

Table 1. Experimental design.

Group	Day of the experiment: 1 to 7	8	9 to 15 Once daily administration of:	12	15	22
A	Adaptation to new conditions	Experimental inoculation with PPMV-1, 10^6 EID_{50}	Aloe vera extract at 300 mg/kg BW[1]	Swabs and organs collection for molecular biology analysis		
B			Aloe vera extract at 500 mg/kg BW[1]			
C			Licorice extract at 300 mg/kg BW[1]			
D			Licorice extract at 500 mg/kg BW[1]			
K			0.9% NaCL			

[1]Body weight.

with the use of the commercial ELISA test kit (IDEXX, Westbrook, ME) according to the method described by Stenzel et al., (2011). Crop swabs and excreta samples also were collected from all birds to screen for parasitic invasions or bacterial infections. The presence of single coccidia oocysts and *Trichomonas gallinae* flagellate parasites was observed. Pathogenic bacteria, PPMV-1, and antibodies against this virus were not detected. Due to a minor risk of parasitic invasions, pigeons were treated with sulfachloropyrazine at 33 mg/1 kg body weight (**BW**) for 3 d and ronidazole at 9 mg/1 kg BW for 7 days. Five d after the end of the therapy, parasitology tests were repeated to determine the efficacy of treatment. The birds were housed in isolated units in a PCL3 biosafety facility of the Department of Poultry Diseases, Faculty of Veterinary Medicine of the University of Warmia and Mazury in Olsztyn. The biosafety facility is equipped with a high efficiency particulate air filtering system and an automated system for pressure control in corridors, bird units, and hygiene stations to prevent contamination of experimental premises. Every group of pigeons was housed in a separate unit. The birds were administered seed mixtures and water ad libitum throughout the experiment.

Experimental design. The experimental design is presented in Table 1. Pigeons were divided into 5 groups of 12 birds each. All pigeons were inoculated oculonasally with 10^6 EID_{50} (50 percent embryo infectious dose) of PPMV-1 at 100 μL per bird (applied to the nostril and the eye at 50 μL each). In the course of 7 d post-inoculation (**dpi**), an aqueous solution of aloe vera extract was administered daily per os at 300 mg/kg BW (group A) or 500 mg/kg BW (group B), and an aqueous solution of licorice extract was administered at 300 mg/kg BW (group C) or 500 mg/kg BW (group D). Control group birds (K) were orally administered 0.9% NaCl. Four, 7, and 14 dpi, cloacal swabs were collected from 4 randomly selected birds in each group with the use of the ESwab Collection and Transport System (Copan Diagnostics, Murrieta, CA). The birds were euthanized, and samples of brain, kidney, liver, and pancreas tissue were collected during an anatomopathological examination.

RNA isolation. Organ samples of 0.2 g were placed in Eppendorf tubes, flooded with 700 μL of sterile PBS, and homogenized in the TissueLyser II disruptor system (Qiagen, Hilden, Germany). The homogenized organs or 450 μL of the liquid from swabs were centrifuged for 15 min at 2,000 x g. The supernatant from the homogenized organs and swabs was collected in the amount of 350 μL for RNA isolation. RNA was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. The concentrations of eluted RNA were measured with the NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA), and the samples were stored at −80°C until further analysis.

Quantitative real-time PCR. The reverse transcription reaction was carried out with the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Before and during the experiment, the presence and quantity of PPMV-1 genetic material in pigeons was determined by TaqMan real-time PCR (**TaqMan qPCR**) with the use of a probe and primers specific for the matrix protein gene, according to the method developed by Wise et al., (2004) and modified by Cattoli et al., (2009). The reaction was performed in the thermocycler (LightCycler® Real-Time PCR System, Roche, Basel, Switzerland) and the mixture had the following composition: 10 μL of TaqMan® Fast Universal PCR Master Mix (Life Technologies, Carlsbad, CA), 1.8 μL of 10 μM (PPMV-1-Forward 5′-AGTGATGTGCTCGGACCTTC-3′ and PPMV-1-Reverse 5′-CCTGAGGAGAGGCATTTGCTA-3′) primers each, 0.24 μL of 25 μM LNA probe (locked nucleic acid) (5′-Hex-GGGAcRgcHtGcTATcC-BHQ-3′ – lowercase letters indicate locked nucleotide position), 3.16 μL of RNase-free water, and 3 μL of cDNA. The reaction was carried out under the following conditions: activation of polymerase at 95°C/30 s, followed by 40 two-stage cycles of: denaturation at 95°C/15 s, and primer annealing and chain elongation at 56°C/60 seconds. The viral copy number and reaction sensitivity were determined by plotting a standard curve. The first stage of the process involved

Table 2. PPMV-1RNA copy numbers in the organs and cloacal swabs collected from the experimental pigeons.

Group	Types of sample														
	Brain			Kidney			Liver			Pancreas			Cloacal swab		
	Day post-inoculation			Day post-inoculation			Day post-inoculation			Day post-inoculation			Day post-inoculation		
	4	7	14	4	7	14	4	7	14	4	7	14	4	7	14
A	1.98^{a}	342.73^{b}	$246.44^{a,b}$	22.96	4460.16	10778.17	7.20^{a}	61.24^{b}	$8.15^{a,b}$	1835.1	194188.08	180.66	0.00^{a}	415.20^{b}	$10.98^{a,b}$
B	10.25^{a}	3586.83^{b}	$3478.70^{a,b}$	64.58	11585.00	18214.55	30.54	53.82	17.96	307.24	225347.30	328452.10	0.00	894.80	6625.86
C	5.58^{a}	2152.14^{b}	$699.36^{a,b}$	$32.96^{a,\dagger}$	10334.82^{b}	$5728.55^{a,b}$	118.76	158.28	5.66	360.86^{a}	175075.90^{b}	$1349.95^{a,b}$	37.97	293.91	13.51
D	3.39^{a}	2346.64^{b}	$208.27^{a,b}$	$25.97^{a,\#}$	8853.93^{b}	$10867.7^{a,b}$	$57.73^{a,b}$	206.26^{b}	10.25^{a}	123.99^{a}	117745.00^{b}	$2700.87^{a,b}$	0.00	416.60	4778.24
K	3.07^{A}	2611.31^{B}	$132.57^{A,B}$	$3.20^{a,*}$	40388.04^{b}	$47603.4^{a,b}$	11.44^{a}	856.69^{b}	$7.21^{a,b}$	2120.51	488860.90	4101.90	0.00	885.34	49.37

^{A,B}Values in the same row with different superscripts differ significantly at $P < 0.01$ in the non-parametric Kruskal–Wallis test for independent samples.

^{a,b}Values in the same row with different superscripts differ significantly at $P < 0.05$ in the non-parametric Kruskal–Wallis test for independent samples.

$^{\#,*,\dagger}$Values in the same column with different superscripts differ significantly at $P < 0.05$ in the Mann–Whitney U test.

the amplification of a 1,195 bp product containing a nucleotide sequence corresponding to the fragment of the gene encoding the PPMV-1 matrix protein and fusion protein, according to the method described by Wise et al., (2004). The amplified fragment contained sequences compatible with the TaqMan qPCR probe and primers. The reaction was carried out in a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany) with the HotStarTaq Plus Master Mix Kit (Qiagen, Hilden, Germany) and the following primers: M629F: 5′-TCGAGICTGTACAATCTTGC-3′ and F581R: 5′-CTGCCACTGCTAGTTGIGATAATCC-3′. The reaction mixture had the following composition: 10 μL of HotStarTaq*Plus* DNA Polymerase (Qiagen, Hilden, Germany), 0.1 μL of 100 μM primers each, 7.8 μL of RNase-free water, and 2 μL of cDNA. The reaction was carried out under the following conditions: 95°C/5 min, followed by 40 cycles of 94°C/1 min, 58°C/1 min, 72°C/1 min, and chain elongation at 72°C/10 minutes. Buffer and nucleotide residues were removed from the resulting product with the Clean-Up kit (A&A Biotechnology, Gdańsk, Poland), and amplicon concentration was measured using a spectrophotometer (NanoDrop 2000, Thermo Scientific, Waltham, MA). The gene copy number was calculated based on amplicon concentration and size with a copy number calculator (University of Rhode Island, Genomics and Sequencing Center, Kingston, RI). Standard 10-fold serial dilutions (initial dilution: 10^8, final dilution: 10^3) of amplicons were used as template DNA.

Statistical analysis. The results were processed in the Statistica PL v. 10.0 program with the use of ANOVA. The Kruskal–Wallis non-parametric test for independent samples was used to determine statistically significant differences among groups (A-K) on each sampling day. The test also was used to evaluate statistically significant differences among sampling days in each group. The Mann–Whitney U non-parametric test was performed to compare 2 independent groups: the experimental group (A-D) and the control group (K). Differences were regarded as significant at $P < 0.05$ and as highly significant at $P < 0.01$.

RESULTS

The experimental infection was mild, and the birds did not present clinical symptoms, except for watery droppings, which were observed from 7 dpi. Deaths were not observed in any of the studied groups. The copy number of PPMV-1 RNA in cloacal swabs and tissue samples collected from birds that were experimentally inoculated with the virus and fed aloe vera and licorice root extracts is presented in Table 2. The amount of paramyxovirus RNA was lowest at 4 dpi and highest at 7 dpi. The above trend was noted in most samples, but the observed differences were not statistically significant in all cases. Significant differences ($P < 0.05$) among sampling d were found in brain samples from groups A-D, kidney samples from groups C, D, and K, liver samples from groups A and K, pancreas samples from groups C and D, and cloacal swabs from group A. The differences in brain samples from group K were highly significant ($P < 0.01$).

The only significant differences ($P = 0.002$) in the viral RNA copy number were found at 4 dpi in kidney samples between experimental groups C and D vs. the control group. In the case of other organs, the PPMV-1 copy numbers were generally higher in organ samples from group K than in organ samples from the experimental groups at 7 dpi. The above trend was most pronounced in group A in which the viral RNA copy number was around 7-fold lower (brain, $P = 0.17$), 9-fold lower (kidneys, $P = 0.30$), and 14-fold lower (liver, $P = 0.16$) in comparison with group K. A similar trend was observed in liver samples from group B relative to group K. In groups C and D, the above trend was less pronounced, but the viral RNA copy number in kidney and liver samples was around 4-fold lower (kidney $P = 0.30$ and liver $P = 0.16$) in comparison with group K. Such correlations were not found in cloacal swabs.

DISCUSSION

The growing prevalence of infectious diseases in animals and the dwindling efficacy of synthetic drugs have

turned researchers' attention to alternative antimicrobial and immune therapies. Recent years have witnessed a growing interest in phytogenic feed additives, which are substances of plant origin used in animal nutrition (Windisch et al., 2008). Phytogenic feed additives are listed in group 2b (natural products – botanically defined) of the European Union Register of Feed Additives, and they have been approved for use in different animal species pursuant to the provisions of Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. The register of feed additives is regularly updated; therefore, the choice of dietary supplements for animals continues to increase. The main advantage of such additives is that they can be administered to animals throughout rearing without the risk of side effects. Therefore, they offer a natural alternative to preventing and treating infectious diseases in the digestive tract and other bodily systems. The use of phytogenic feed additives in livestock, including poultry, has been extensively studied; however, there is a general scarcity of information about phytogenic feed additives for pigeons. Domestic pigeons are bred mainly for competitive sport performance (carrier or racing pigeons) and as ornamental birds. National and international competitions and shows contribute to the spread of infectious diseases in pigeon populations. Complex diseases with a mixed etiology, including viral (mostly PiCV), pose a much more serious problem in pigeon breeding than single infections (Raue et al., 2005). All of the above considerations prompted the development of an experimental model in which pigeons were infected with a pigeon NDV strain that could be easily cultured and standardized under laboratory conditions. The infected birds were administered plant extracts with potentially antiviral properties. The experimental inoculation induced a PPMV-1 infection in the examined birds, but watery droppings were the only clinical symptoms that were observed from 7 dpi. Our findings are partially consistent with the results reported by other authors. Dortmans et al., (2011) failed to induce the clinical symptoms of disease in pigeons experimentally infected with PPMV-1 (strain AV324/96; intracerebral pathogenicity index (**ICPI**): 0.2), but reported viral shedding. Śmietanka et al., (2014) observed neurological symptoms and reported deaths in pigeons experimentally infected with PPMV-1. However, the above authors used a different viral strain (PPMV-1/pigeon/Poland/332/05; ICPI: 1.05). The observed discrepancies could be attributed to differences in viral properties, viral strains, inoculum dose, and the route of experimental inoculation. The mild form of the experimental infection (absence of deaths) and the presence of watery droppings as the only clinical symptom probably resulted from the tropism of the viral strain used in the study. This hypothesis was confirmed by the results of quantitative real-time PCR, which revealed larger amounts of PPMV-1 genetic material in the kidneys and the pancreas than in the brain. In our experiment, PPMV-1 RNA copy numbers in pigeon organs were somewhat similar to those reported by Śmietanka et al., (2014) in whose study the highest copy number also was found in kidneys at 14 dpi. However, the results noted in other tissues, in particular the brain, clearly differed from our findings. In the work of Śmietanka et al., (2014), viral RNA copy numbers were highest in brain samples at 4 and 7 dpi, which was consistent with the emergence of neurological symptoms in infected birds. In our experiment, the amount of PPMV-1 genetic material was highest in pancreas samples, which can probably be attributed to differences in viral tissue tropism. In our study, PPMV-1 replicated most readily at 7 dpi, and least rapidly at 4 dpi. In most cases, the differences between these dates were statistically significant.

A comparison of viral RNA copy numbers in the organs of infected pigeons receiving plant extracts and control group pigeons revealed lower copy numbers of PPMV-1 RNA in inoculated pigeons fed an aloe extract dose of 300 mg/kg BW. In this group, viral RNA copy numbers were around 14-fold lower in liver samples, 9-fold lower in kidney samples, and 7-fold lower in brain samples than in control at 7 dpi. A clear trend also was observed in the livers of pigeons administered aloe extract at 500 mg/kg BW relative to control group birds. The observed differences were not significant due to relatively high variations among sample values, which contributed to high standard deviation. Despite the above, the noted trend suggests that aloe extract is capable of inhibiting PPMV-1 replication in a dose-dependent manner. There is a general scarcity of published information about the immunomodulatory effects of aloe vera extract in pigeons. Shokraneh et al., (2016) evaluated the performance and selected immune parameters of chickens infected with NDV and receiving aloe vera extract. However, the cited authors did not analyze viral RNA copy numbers in the organs of infected birds; therefore, our results cannot be compared with their findings. According to the literature, aloe vera extract not only improves performance (Shokraneh et al., 2016), but also alleviates the symptoms of experimental infection and lowers mortality, which suggests that aloe vera has a potentially antiviral effect on NDV (Waihenya et al., 2002).

Licorice extract is a phytogenic feed additive that also demonstrates antiviral activity against NDV (Omer et al., 2014). In this experiment, licorice extract was a less potent viral inhibitor than aloe vera. However, the viral RNA copy number in livers and kidneys of pigeons receiving licorice extract was around 4-fold lower than in the control group, which suggests that licorice exerts antiviral effects.

An absence of similar relationships in cloacal swabs can be largely attributed to the fact that the protocol for extracting nucleic acids from this type of clinical material is difficult to standardize in quantitative analyses. Cloacal swabs from various individuals differ in the quantity of genetic material. Incubation of swabs

in the same quantity of liquid is probably insufficient to produce comparable results between individuals. In tissue samples, this is a less important consideration because sections have the same weight and are collected from the same location in the analyzed organ; therefore, they contain comparable numbers of cells with PPMV-1 genetic material. For this reason, the results of cloacal swab analysis are doubtful and difficult to compare.

The results of this study indicate that both aloe vera and licorice extracts inhibit PPMV-1 replication in pigeons. The aloe vera dose of 300 mg/kg BW was characterized by the highest inhibitory activity. Our findings suggest that aloe vera and licorice extracts can be used as feed additives during supportive treatment of viral diseases in pigeons. The mode of action of aloe vera and licorice extracts has not been fully elucidated in any bird species; therefore, further research is needed to determine the influence of these feed additives on selected immune parameters in pigeons infected with PPMV-1.

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