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APPLICATION OF YOLK IMMUNOGLOBULINS (IGY) IN THE TREATMENT OF SOME INFECTIOUS DISEASES OF DOGS

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Abstract

This article discusses the development of IgY technologies for the treatment and prevention of infectious diseases in dogs, such as parvovirus enteritis, distemper and hepatitis. These diseases are a significant threat to canine health and they are highly lethal, that emphasizing the need for new, effective and safe agents. Methods for isolating, purifying and determining the concentration of IgY immunoglobulins from egg yolks of hyperimmunized chickens are described. Clinical tests on dogs to determine the efficacy of the resulting preparation were also performed. During the study it was found that the IgY-based product significantly improves the immune response in dogs, helps to reduce the severity of the parvovirus enteritis, canine distemper, and infectious hepatitis and accelerates the dog's recovery by 2 days (20-28%). Potential advantages of the new drug in comparison with existing therapeutic agents, as well as its possible application in veterinary practice are discussed. The proposed IgY-based drug is a prospective tool for the treatment and prevention of serious infectious diseases in dogs, which opens up new opportunities in the field of veterinary medicine.

Keywords: IgY technologies, Parvovirus enteritis, Canine distemper, Infectious hepatitis

1. Introduction

Dogs are one of the common pets of densely populated areas. Most of them are susceptible to many infectious diseases. If they are not properly treated, the chance of survival is minimal. While most domestic dogs are vaccinated by their owners, stray dogs do not have this option, so they have a higher mortality rate from infectious diseases [1].

The canine infectious diseases are a group of diseases caused by various pathogens such as bacteria, viruses, fungi or parasites. One of these are parvovirus enteritis, distemper, infectious hepatitis [2].

Parvovirus enteritis, the causative agent of which is canine parvovirus (CPV), causes acute inflammation of the intestines manifested by vomiting, diarrhea, anorexia, and dehydration. Parvovirus enteritis is particularly dangerous for puppies and it can be fatal [3].

Canine distemper, the causative agent of canine distemper virus (CDV), is an infectious disease characterized by high contagiousness and it can affect various organs including the nervous system, respiratory tract, digestive system and skin. Symptoms can range from fever and cough to neurologic symptoms and death [4].

Canine hepatitis, also known as infectious hepatitis, is an acute viral disease caused by Canine Adenovirus Type 1 (CAV-1). This virus attacks liver cells and it may also affect the vascular system, kidneys,

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and eyes. Canine hepatitis is usually transmitted through contact with infected dogs or through their secretions such as saliva, urine, and feces [5, 6].

Discovering alternative treatments for infectious diseases of dogs is an important aspect of veterinary medicine for several reasons. Many existing methods of treatment have disadvantages and limitations. For example, the usage of antibiotics can lead to the development of bacterial resistance, which make them less effective at fighting with infection [7]. In addition, some drugs may have side effects or be toxic to the dog's body [8].

The availability of modern therapies can be limited due to their high cost. For some dog owners, the cost of medical treatment may be too high, therefore preventing access to quality medical care for their animals. Vaccination of dogs against infectious diseases is not always available for homeless or domestic dogs. In addition, some owners may refuse to get vaccinated their pets for a variety of reasons.

Thus, finding alternative treatments for canine infectious diseases is critical to providing effective and affordable treatment for all dogs, including stray ones. Immunoglobulins of group Y (IgY) are a class of antibodies that are present in amphibians, reptiles and birds. The application of IgY technologies has great potential in various fields and continues to attract attention of researchers and industry due to their unique properties and perspectives for utilization. IgY-related technologies are an important tool for infectious disease control, medical, veterinary and scientific researches, as well as for the development of new methods for the diagnosis and treatment of various pathologies [9].

One of the main advantages of avian antibodies over IgG is the ability to produce a significant quantity of antibodies. One chicken can produce 15-17 times more antibodies in a month than a rabbit. This makes the process of IgY production more efficient and economically beneficial [10].

Avian antibodies have 5 times higher affinity for a particular antigen and react faster than mammalian IgG, which make them more effective in recognizing and neutralizing pathogens [11].

IgY is less immunogenic than IgG because it is produced in bird eggs and does not elicit as strong an immune response in the host. IgY do not interact with complement components, rheumatoid factor and Fc-receptors of mammalian cells, compared to IgG immunoglobulin, which makes them safe for use in humans and animals thereby reducing the possibility of undesirable side effects [12].

IgY can be readily obtained from the eggs of birds such as chickens and its production does not require complex technology or expensive equipment, that making it more accessible and cheaper to produce than IgG. The process of IgY isolation occurs through the laying of eggs, which is bloodless and physiologically safe for the animal, unlike the process of IgG production, which requires bloodletting and can cause stress and pain for the animal [13].

Based on the above-mentioned facts, we set a goal — creation of a complex remedy based on immunoglobulins of Y type specific to parvovirus, distemper and canine hepatitis.

2. Material and methods

To obtain eggs with high quantity of immunoglobulins specific to pathogens of a number of diseases, the method of hyperimmunization of hens with *Nobivac* vaccine was used. After collection, eggs were washed in running water for cleaning shells. The yolks were dissolved in distilled water at a ratio of 1:8, using a Magnetic Hot Plat model 78-1 for obtaining a homogeneous solution [14].

The mixture was poured into flasks and left in a freezer at -20 ℃. The next day, it was left to thaw at room temperature. After complete thawing, the yolk-water mixture was manually shaken and poured into flasks for centrifugation (Fig. 1). Centrifugation was carried out at 10000 rpm for 15 min at 4℃ on a Celesta Centrifuger BLT with a cooling box. The supernatant of the obtained liquid was separated and salinized in ammonium sulfate $(NH_4)_2SO_4$ at a 1:1 ratio. After overnight in the refrigerator, the liquid was centrifuged again under the same conditions. The lower fraction of the liquid was placed in MD25 dialysis bags into 5-liter beaker for dialysis on distilled water (during 24 hours 2-time water replacement). The magnetic stirrer mentioned above was used to accelerate the process.

The selection of dogs was performed by veterinarians at AARC International Veterinary Hospital and Bona Vet Clinic. The medical history of animals was compiled taking into due account such data as age, breed and sex of the dog, as well as the date of admission.

To verify the diagnosis, the animals were subjected to a rapid test "Canine Parvovirus Antibody test kit" for the presence of parvovirus antigens based on the immunochromatographic method. Animal feces served as samples. The rapid test "Distemper Antibody test kit" for the detection of canine distemper virus detected the presence of the virus according to the same principle as the rapid test for parvovirus enteritis. Infectious hepatitis of dogs was confirmed by real time PCR, the material for the study was swabs from the mucous membranes of the nasal cavity.

Fig. 1. The process of isolation and purification of immunoglobulins. A — Hyperimmunization of chickens with the polyclonal attenuated vaccine *Nobivac*. B — Eggs of hyperimmunized chickens; C – Homogenization of egg yolks; D — PerkinElmer® Lambda Bio+ spectrophotometer; E – Staining of a control sample with immunoglobulins with Bradford reagent; F — Determination of the concentration of the isolated immunoglobulins.

Eight dogs aged from 4 to 9 months with parvovirus enteritis, distemper, and hepatitis were included in the study (Fig. 2). Two treatment groups were analyzed: maintenance therapy (n=4) and maintenance therapy with the addition of avian immunoglobulins against the pathogens of the mentioned diseases (n=4). Maintenance therapy against Hepatin included: infusion of ringer lactate (50 ml solution with 50 ml NaCl), subcutaneous injection of 0.5 ml gentamicin sulfate 10%, ademetionine 400 mg orally ½ tablet once daily, 0.5 ml methiclopramide intramuscularly, 1 ml furosemide intramuscularly, 0.5 ml ketonal intramuscularly, and feeding with Hepatin pet food and pate.

Maintenance therapy for canine distemper included: infusion of 50 ml Ringers' lactate with 50 ml NaCl, 1 ml vitamin C, 0.5 ml vitamin B, 10 ml metronidazole, intramuscular injection of 1 ml ceftriaxone and novocaine solution, intramuscular injection of 0.5 ml methiclopramide, subcutaneous injection of 0.5 ml meloxicam, 3 ml enterogermina oral suspension orally during the day and 3 ml in the evening. Nutrition: feed and pate "Gastrointestinal". Maintenance therapy for parvovirus enteritis: infusion of 50 ml of Ringer's lactate, 50 ml of NaCl, 20 ml of metronidazole, intramuscular injection of 0.5 ml of 20% enroflaxacin, intramuscular injection of 0.5 ml of methiclopramide, oral administration of 40 mg of pantoprazole, 2 ml of enterogermina suspension by day and 2 ml in the evening orally. Feeding: "Gastrointestinal", pate or pet food.

In addition to maintenance therapy, dogs in the IgY group were orally injected with 150 ml of IgY solution from the first day of study, twice a day (morning and evening) on an empty stomach, for five days. In parallel, the dogs were rinsed nasally with 10 ml of antibody solution twice a day by pipette.

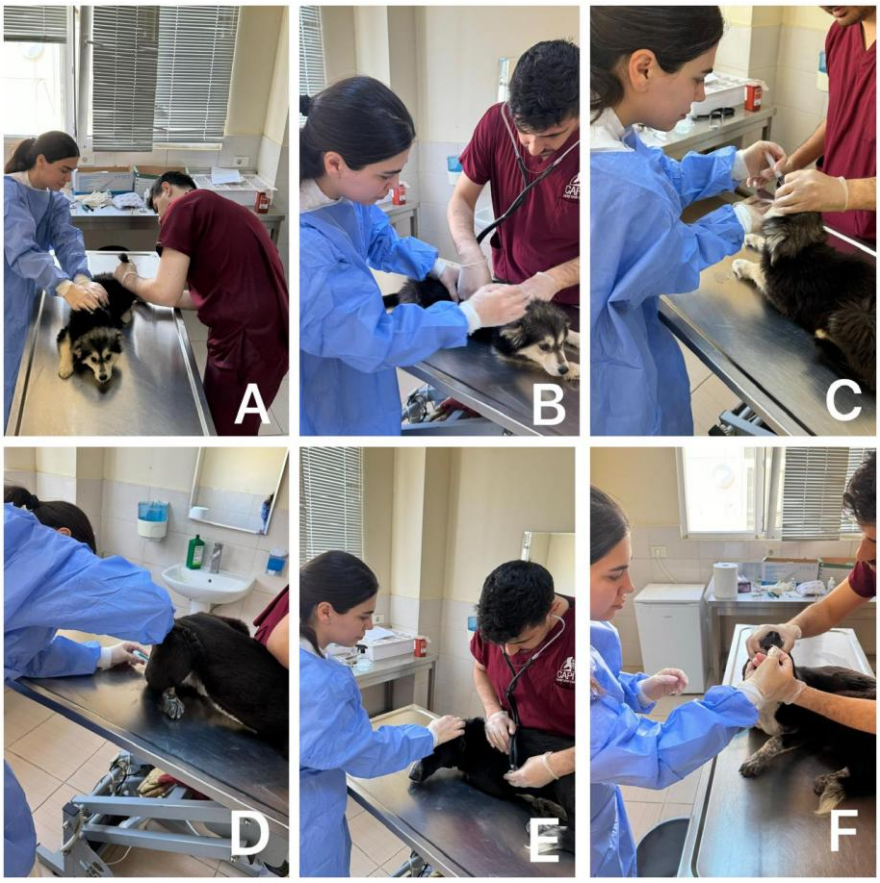

Fig. 2. Clinical test of immunoglobulin Y preparations for the treatment of distemper, hepatitis, parvovirus enteritis in dogs. A, B, D, E – physical examination of dogs; C, F – oral administration of immunoglobulins Y.

A sum of 1600 ml of IgY solution were orally injected in each dog in this group during the whole treatment period. Careful observation of the dogs' well-being and clinical picture of the disease was carried out every 12 hours.

3. Results and discussion

The volume of the obtained IgY solution was on average 480.3±94.8. The concentration of it was on the mean 52.6±3.9 mg/ml.

According to the data of Table 1 all experimental animals survived. It is possible to observe gradual recovery of sick dogs during 7-8 days using the classical maintenance method of treatment.

Table 1. Observation of dogs from the first group (maintenance therapy or control group)

Health state	**Dog No. 1 (parvovirus enteritis)**	**Dog No. 2 (parvovirus enteritis)**	**Dog No. 3 (hepatitis)**	**Dog No.4 (distemper)**
Day 1	Extremely severe state: hypothermia; fever 40-41°C (temporary has risen to 42°C); tachycardia;	Extremely severe state: tachycardia; fever 41°C; diarrhea; blood-stained vomiting; lethargy; apathy; dehydration; lack of	Extremely severe state: yellowing of the mucous membranes of the eyes, gums and skin; clouding of the	Extremely severe state: fever 41°C; coughing; inflammation of the tonsils; lethargy; dehydration; loss of

	dehydration; frequent episodes of diarrhea with admixture of blood; vomiting; lethargy; lack of appetite; lack of mobility	appetite; immobility; drowsiness.	cornea of the left eye; fever 42°C; frequent vomiting and diarrhea; tachycardia, lethargy; lack of appetite	appetite; severe diarrhea; vomiting with remnant food and bile; mucous secretions from the nose; enlargement of the lymph nodes
Day 2	Major severe state: hypothermia - body temperature 40-40,5°C, has risen to 41°C; diarrhea without blood admixture; vomiting; lack of appetite; drowsiness; lethargy	Major severe state: body temperature rise to 42°C up but it has decreased to 39.8°C; diarrhea (9 times per day); lethargy; vomiting (4 episodes); lack of appetite	Extremely severe state: yellowing of the mucous membranes of the eyes, gums and skin; fever 42°C, frequent vomiting and diarrhea, tachycardia, lethargy, lack of appetite	Major severe state: fever 40-41°C, has decreased to 38°C toward evening, then rose again; cough; lymph nodes in the neck and submandibular fossa are enlarged; diarrhea (7-9 times a day); vomiting (3 times); lack of appetite; noticeable redness of the eyes; the quantity of mucous secretions from the nose decrease
Day 3	Moderate severe state: body temperature 37.9°C; mild diarrhea; 2 vomiting attacks; demonstration of interest to the surrounding; little activity.	Moderate severe state: temperature is normal (37.7°C), frequent episodes of diarrhea (6 times per day); lack of appetite; lethargy, however, motor activity appeared; vomiting is absent.	Major severe state: temperature is normal; no tachycardia; no vomiting episodes except once in the evening; no diarrhea; jaundice of mucous membranes continues to persist	Major severe state: body temperature is 38-39 °C; tachycardia is absent; coughing; lymph nodes are not palpable; episodes of diarrhea continue; there are no episodes of diarrhea; inflammation of tonsils is not observed; redness of the eyes continues to persist, but intensity is noticeably reduced; the amount of mucous secretions decrease
Day 4	Moderate severe state: temperature is normal (37.8°C); mild diarrhea (5 episodes); vomiting (1 time after meal).	Moderate severe state: temperature is normal (37.5°C); appetite is present; vomiting after two meals (2 times); mild diarrhea (4 times).	Moderate severe state: jaundice of mucous membranes starts to subside; however, it still persists	Moderate severe state: Coughing; eye irritation is reducing slightly; however, it persists; rare episodes of diarrhea (4 times after meals), presence of appetite
Day 5	Minor severe state: appetite is present; vomiting after meals; body temperature in normal range; diarrhea (3 times a day)	Minor severe state: rare episodes of diarrhea (3 times); relative increased activity; appetite is present; increasing of fluid intake	Moderate severe state: the jaundice of the mucous membranes reduces significantly; however, it still persists. The opacity	Minor severe state: redness; eye irritation; rare episodes of diarrhea; no vomiting; rare episodes of coughing. Nasal mucous

			in the cornea of the eyes decreases	secretions have decreased.
Day 6	Minor severe state: mild diarrhea (5 episodes); body temperature is normal; active; appetite is present	Minor severe state: rare episodes of diarrhea (3 times); increasing of activity; increasing of fluid intake	Minor severe state: corneal opacity of the eyes is significantly reduced; jaundice of the mucous membranes has decreased	Minor severe state: nasal secretions significantly decrease; appetite is present; eye irritation has decreased; redness; rare episodes of diarrhea and coughs
Day 7	Minor severe state: rare episodes of diarrhea (3-4 times)	Recovery	Recovery	Minor severe state: presence of appetite; active; rare episodes of diarrhea; mucous discharge from the nose is absent; the eyes have acquired the previous coloring of the white coating; cough is absent.
Day 8	Recovery			Recovery
Totally: 8 days				

In the next table (Table 2) the same tendency is observed, however, at an accelerated rate. In case of application of complex treatment (supportive therapy together with IgY technologies) full recovery of dogs was achieved on 5-6 days.

Table 2. Monitoring of the second group of dogs that received maintenance therapy in association with oral and nasal administration of IgY.

Health state	**Dog No. 1 (parvovirus enteritis)**	**Dog No. 2 (parvovirus enteritis)**	**Dog No.3 (distemper)**	**Dog No. 4 (hepatitis)**
Day 1	Extremely severe state: body temperature 40-42°C; tachycardia; dehydration; frequent episodes of diarrhea; vomiting; lethargy; lack of appetite; immobility	Major severe state: body temperature 42-42.5°C; tachycardia; diarrhea; vomiting; lethargy; apathy; dehydration; lack of appetite; immobility; drowsiness	Extremely severe state: body temperature 41-42°C; lethargy; dehydration; lack of appetite; severe diarrhea; vomiting with food residues and bile; conjunctivitis; mucous nasal discharge; enlarged lymph nodes; coughing.	Extremely severe state: Yellowing of mucous membranes of eyes, gums and skin; temperature rose to 42°C and decreased to 38°C by the evening; increased vomiting and diarrhea; tachycardia; lethargy; lack of appetite

Day 2	Major severe state: hypothermia; moderate diarrhea; vomiting (3 episodes); lack of appetite; drowsiness; lethargy; body temperature 40°C	Moderate severe state: diarrhea (9 times per day); lethargy; vomiting (4 episodes); lack of appetite; body temperature 41.3°C	Major severe state: temperature 40°C, in the evening it decreased to 38°C; lymph nodes in the neck and submandibular fossa are enlarged; diarrhea (7-9 times a day); vomiting (3 times); lack of appetite; conjunctivitis; the amount of mucous discharge from the nose decreased.	Major severe state: temperature 40°C; no vomiting; short-term diarrhea (4 episodes); jaundice of mucous membranes
Day 3	Moderate severe state: body temperature 38°C (normal); mild diarrhea; appetite is present; no vomiting, showing interest to surrounding; active	Minor severe state: body temperature 37.5°C (normal); episodes of diarrhea (6 times per day); appetite is present; vomiting (after one of the meals).	Moderate severe state: body temperature is normal; no tachycardia; lymph nodes are not palpable; episodes of diarrhea; appetite is present; there were two episodes of vomiting after meals; the redness of the eyes; the quantity of mucous secretions has decreased	Moderate severe state: normal temperature; no tachycardia; no vomiting except once in the evening; no diarrhea; jaundice of mucous membranes
Day 4	Minor severe state: temperature 37.7 (normal); mild diarrhea (3 episodes); vomiting; tachycardia; appetite is normal; active.	Minor severe state: normal temperature, good appetite; active; no vomiting; rare episodes of diarrhea.	Minor severe state: eye irritation has reduced; eye redness has decreased; rare episodes of diarrhea (4 times); no vomiting; dog is alert; appetite is present	Minor severe state: the jaundice on the mucous membranes has subsided.
Day 5	Recovery	Recovery	Recovery. Minor eye redness	Recovery
Day 6			Recovery	
Totally: 6 days				

In experiments on animals the effectiveness of IgY-based compound in the treatment of infectious diseases of dogs (parvovirus enteritis, plague, hepatitis) was demonstrated. The product reduced the severity of clinical symptoms, decreased the viral load and promoted rapid recovery of dogs affected by distemper, hepatitis or parvovirus enteritis. This is due to neutralization of viruses and bacteria, enhancement of immune response, optimization of regenerative processes.

Thus, oral and nasal injection of IgY type immunoglobulins significantly accelerated the therapeutic effect against parvovirus enteritis, distemper, and hepatitis. It can be concluded that the developed IgY-

based product is a perspective agent for the treatment of infectious diseases of dogs. Its efficiency has been proved on various models of infectious diseases, which confirms its potential for practical application [15].

The developed drug has the potential to become an effective remedy in the treatment of infectious diseases in dogs. The presented investigation could also have important implications for the development of drugs for infectious diseases in other animal species and, in human [15,16,17].

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