



RESEARCH ARTICLE

Ameliorative effect of Panchagavya on Newcastle Disease in Layer Chicken

A Sumithra, P Srinivasan*, GA Balasubramaniam, TR Gopalakrishna Murthy and P Balachandran

Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

ARTICLE INFO

Received: February 18, 2013

Revised: February 22, 2013

Accepted: February 24, 2013

Key words:

Clinical manifestation

Layer chicken

Newcastle disease

Panchagavya

ABSTRACT

The Ameliorative effect of panchagavya on clinical manifestation of Newcastle disease in layer chicken was assessed. Panchagavya was incorporated in the basal diet at three levels (0.5, 0.6 and 0.7 per cent) and fed to six groups (T₃ to T₈) and two groups (T₁ to T₂) were served as controls. Vaccination against Newcastle disease was carried out in 4 groups (T₂, T₆, T₇ and T₈) and the remaining four groups (T₁, T₃, T₄ and T₅) were not vaccinated. The mean HI titre values at 16 wk of age in Panchagavya fed and vaccinated group (T₆, T₇ and T₈) showed significant difference with vaccinated group (T₂). The unvaccinated control (T₁) and Panchagavya fed and unvaccinated groups (T₃, T₄ and T₅) showed nil titre. All these groups were challenged with virulent Newcastle disease virus (NDV) at the age of 17th week. The incubation period was short in unvaccinated control group (T₁) compared to other groups (T₂ to T₈). The birds of unvaccinated control group (T₁) and Panchagavya fed and unvaccinated groups (T₃, T₄ and T₅) showed dullness, depression, loss of appetite, gasping, inhalation through half opened mouth, ocular-nasal discharge, partial or complete closure of eyes, greenish watery diarrhea and sudden death. In vaccinated control group (T₂), dullness, depression and anorexia with greenish watery diarrhea were observed in nine out of twelve birds where as in Panchagavya fed and vaccinated groups (T₆ and T₇), showed similar symptoms in two out of twelve birds. Highest (100%) and lowest (8.3%) mortality were observed in unvaccinated control (T₁) and Panchagavya fed and vaccinated groups (T₆ and T₇), respectively. No mortality was observed in T₈ as compared to 16.6 per cent mortality in T₂ group. The haemagglutination activity in pooled organ samples from dead birds of different groups revealed high titre in vaccinated (T₂) as well as vaccinated and panchgavya supplemented groups (T₆ and T₇) compared to other groups. From the above experiment, it was concluded that Panchagavya minimized the clinical manifestation and severity of Newcastle disease in layer chicken when used along with Newcastle disease immunization programme.

*Corresponding Address:

P Srinivasan

srinipat2004@yahoo.com

Cite This Article as: Sumithra A, P Srinivasan, GA Balasubramaniam, TRG Murthy and P Balachandran, 2013.

Ameliorative effect of panchagavya on Newcastle disease in layer chicken. *Inter J Agri Biosci*, 2(2): 60-63.

www.ijagbio.com

INTRODUCTION

Poultry farming is always prone to a heavy risk of increased disease incidences leading to high mortality even after scheduled mass vaccination. Sustained economically viable poultry production demands the stringent control of various infectious diseases affecting the birds which incur huge economic losses to the poultry farm owners. Among the infectious diseases, Newcastle disease is a deadly viral disease of poultry due to its high contagiousness and rapid spreading among chicken and

other domestic and semi-domestic species of birds. Newcastle disease vaccination is routine in commercial chicken flocks in most Asian countries. It is known that vaccination of poultry provides an excellent means to lessen clinical signs of infection caused by virulent Newcastle disease virus (NDV) (Kapczynski and King, 2005). Large number of reports are available on outbreak of Newcastle disease (ND) resulting in alarming economic losses mainly due to vaccination failure state even after programmed vaccination schedules have been used (Chakraborty and Chatterjee, 1998). To overcome these

conditions modulation of microenvironment of the immune system seems to be essential. This can be achieved by immunomodulator or immunostimulating compounds. Immuno-modulators by definition are inherently non-specific in nature as they are envisaged to enhance body resistance against infection. They can act through innate and adoptive immune response. Immunomodulation of bird may lead to increased antibody production, increased cellular immune response and increased macrophage phagocytic activity which positively correlates with enhanced resistance to various viral and bacterial infections (Liu, 1999).

Panchagavya is a term used to describe five major substances, obtained from cow, which include cow's urine, milk, ghee, curd and dung. All the five substances possess medicinal properties against many disorders and are used for the medicinal purpose singly or in combination with some other herbs (Dhama *et al.*, 2005). It also has antibiotic, antifungal and antiviral effects in addition to increasing bioavailability of other medicines (Natarajan, 2003). Panchagavya have been used in many applications as growth promoter in poultry industry (Mathivanan *et al.*, 2006; Sumithra *et al.*, 2011). Though considerable literature is available on Panchagavya and its therapeutic use in human and laboratory animals, there is no proved data of its antiviral efficacy in poultry. Hence the present study was undertaken to assess the ameliorative effect of Panchagavya on clinical manifestations of Newcastle disease in layer chicken.

MATERIALS AND METHODS

Preparation of Panchagavya

The Panchagavya was prepared by using cow dung -5 parts, urine -3 parts, milk -2 parts, curd -2 parts, ghee -1 part, sugarcane juice -3 parts, tender coconut water -3 parts, ripened banana -12 Nos, and yeast powder -250 mg. as per the method described by Natarajan (2003). The dung, urine, milk, curd and ghee were obtained from indigenous cow. The fresh dung was thoroughly mixed with ghee in a wide mouth mud pot and kept for three days. The above mixture was thoroughly mixed once a day. On the fourth day, other ingredients were added to the contents of the mud pot, mixed properly, and covered with nylon net to prevent the entry of flies. This preparation was kept in shaded place and subjected to thorough, uniform mixing twice a day. The above mixture was allowed for fermentation up to 21 days.

Experimental birds

Ninety six, day old White Leghorn cockerels were obtained from a commercial hatchery. They were weighed, wing banded and randomly divided into eight treatment groups (T₁ to T₈) each containing twelve birds. All the chicks were reared in cage system under standard managerial conditions throughout the experimental period of 20 wks. The experimental diet was formulated according to the standards of Bureau of Indian Standards (BIS) specifications (1992). Panchagavya was incorporated into the basal diet at three different levels (0.5, 0.6 and 0.7 per cent) as indicated in Table 1.

Immunization programme

Birds belonging to the group T₂, T₆, T₇, and T₈ were vaccinated against Newcastle disease on the seventh day (RDVF₁ strain) and 35th day (LaSota strain) through ocular-nasal route. Revaccination was done with RDVK and inactivated killed vaccine on 8th and 14th wk respectively through subcutaneous route. All the birds were vaccinated against infectious bursal disease virus at second and third wks of age. Serum monitoring of antibody level against Newcastle disease was done by using a modified filter paper technique as described by Brugh and Beard (1980).

Challenging the experimental birds with NDV

The experimental birds of both vaccinated and unvaccinated groups were challenged with field isolate of velogenic Newcastle disease virus (VNDV) at the dose rate of 0.1 ml of 10^{8.5} CID per bird, through ocular-nasal route at the age of 17th wk. Then the birds were maintained for the period of 3 wks. All the birds were examined twice a day for onset and severity of clinical signs (early morning and late evening) and number of birds died in different treatment groups.

Newcastle disease viral antigen detection

The hemagglutination (HA) titer of the NDV virus was measured in pooled organs sample (spleen, caecal tonsil, proventriculus, lung and trachea) of each dead bird belonging to different treatment groups in 96-well microplates with V-shaped bottom. The sample was serially diluted in a twofold dilution with PBS. Into each well containing 50µl of the sample solution, an equal volume of one percent chicken erythrocytes was added. Following mechanical vibration, the plates containing the mixture of sample and erythrocytes were kept at room temperature, and the results were recorded after 30 min. The titer was

Table 1: Experimental design, Serum antibody titer, Mortality pattern and Haemagglutination titer of cockerels fed with Panchagavya and challenged with virulent Newcastle disease virus (VNDV)

S. No	Group	Number of birds	Basal diet	Panchagavya level (%)	Vaccination (7 th , 35 th day and 8 th and 14 th wk)	Mean HI titer at 16 th Wk	Challenging with VNDV (17 th week)	Mortality (%)	Mean HA titer
1	T ₁	12	+	-	-	-	+	100	6.75
2	T ₂	12	+	-	+	8.42	+	16.6	8.50
3	T ₃	12	+	0.5	-	-	+	91.66	6.45
4	T ₄	12	+	0.6	-	-	+	83.33	6.30
5	T ₅	12	+	0.7	-	-	+	75.00	5.77
6	T ₆	12	+	0.5	+	9.00	+	8.30	8.00
7	T ₇	12	+	0.6	+	9.33	+	8.30	8.00
8	T ₈	12	+	0.7	+	9.66	+	-	-

expressed as the reciprocal of the highest dilution of the sample showing complete hemagglutination. The assay was triplicated for each sample dilution, and the HA titer determined represents the titer identically recorded with all of the three or two out of the three tests. Confirmation of NDV in HA positive samples was determined by haemagglutination inhibition (HI) test with specific NDV antiserum (Alexander, 1989).

RESULTS AND DISCUSSION

Serum antibody titre in vaccinated birds

The mean (\pm S.E) HI titre values at 16 wk of age were 8.42 ± 0.19 , 9.00 ± 0.21 , 9.33 ± 0.22 and 9.66 ± 0.22 for the groups T₂, T₆, T₇ and T₈ groups, respectively (Table 1). Comparison of treatment means revealed highly significant ($P < 0.01$) differences between vaccinated groups. Among the vaccinated groups, T₆, T₇ and T₈ showed significant ($P < 0.01$) difference with group T₂. The unvaccinated control (T₁) and Panchagavya fed and unvaccinated groups (T₃, T₄ and T₅) showed no titre indicating the absence of antibodies against NDV. This finding concurs with the earlier observations of Singh and Chauhan (2000), Gupta (2003) and Dhama *et al.* (2005) who also reported that Panchagavya or Panchagavya with herbal formulation or Panchagavya components individually or in combination increased both the humoral and cell mediated immunity in animals.

Incubation period in VNDV challenged birds

The incubation period of experimental NDV infection varied from 2 to 5, 5 to 14, 4 to 9, 5 to 10, 5 to 10, 7 to 13, 7 to 13 and 8 to 12 DPI for the groups T₁ to T₈. The two of the three Panchagavya fed and unvaccinated groups (T₄ and T₅) and another two of the three Panchagavya fed and vaccinated groups (T₆ and T₇) showed same incubation period as 5 to 10 and 7 to 13 days respectively. On comparison, the incubation period was shorter in T₁ than T₈ group. The incubation period was short in unvaccinated control (T₁) group compared to vaccinated control (T₂), Panchagavya fed and unvaccinated (T₃, T₄ and T₅) and Panchagavya fed and vaccinated (T₆, T₇ and T₈) groups. This finding is in accordance with the report of Parede and Young (1990) and Hamid *et al.* (1991). They also observed short and long incubation period in unvaccinated and vaccinated birds respectively.

Clinical manifestation and mortality in VNDV challenged birds

The birds of unvaccinated control group (T₁) showed dullness, ruffled feathers, depression, marked reduction in feed and water consumption by 2 DPI, sneezing, gasping, inhalation through half opened mouth, ocular-nasal discharge, cyanosis of comb, partial or complete closure of eyes and prostration by 3 DPI. Greenish watery diarrhoea was noticed on 2 DPI, which intensified on 5 DPI and then persisted up to 9 DPI. Shivering and sternal recumbences were noticed on 4 DPI. Sudden death was noticed in one bird on 3 DPI and two birds on 4 DPI. The characteristic sign exhibited in this group is attributed to their lower resistance as they were not previously vaccinated against ND and all are died from NDV within 9 DPI resulting in 100 per cent mortality, which is in

agreement with the finding of Parede and Young (1990); Hamid *et al.* (1991) and Pazhanivel *et al.* (2002).

In vaccinated control group (T₂), dullness, depression and anorexia with greenish watery diarrhoea were noticed in 9 out of 12 birds from 5 to 14 DPI. Four birds showed gasping, oculo-nasal discharge, subsequently two died and the remaining two were recovered with a mortality percentage of 16.6 (2/12). Findings of the present study indicates that the vaccination protects the birds from more serious consequences of disease, but viral replication and shedding may still occur although at a reduced level and causes mortality in few birds. These observations are in accordance with that of Hamid *et al.* (1991) and Pazhanivel *et al.* (2002), they also observed depression, dullness and mild respiratory signs in birds inoculated with viscerotropic velogenic Newcastle disease virus (VVNDV).

In Panchagavya fed and unvaccinated groups (T₃, T₄ and T₅) birds showed dullness, anorexia and depression on 4 and 5 DPI in T₃ and T₄, T₅ groups respectively. Greenish watery diarrhoea was observed from 4 to 16 DPI in T₃ and 5 to 15 DPI in T₄ and T₅ groups. Respiratory signs similar to that of T₁ were observed in majority of the birds. Mortality percentage in T₃, T₄ and T₅ were 91.66 (11/12), 83.3 (10/12) and 75 (9/12) respectively. The result indicates that the Panchgavya may not have virucidal effect; however the dose response study showed that the clinical manifestation and mortality was reduced in a dose dependent manner. Chauhan and Singh (2001) and Chauhan *et al.* (2001) reported immune-modulatory properties of cow urine one of the constituent of Panchagavya in mice. They recorded an increase in humoral and cellular immunity of 45 and 59 per cent respectively, in mice treated with cow urine. The cow urine also stimulated the production of interleukin 1 and 2 by 16 and 21 per cent respectively, from peripheral blood leucocytes of mice. The phagocytic activity of macrophages was enhanced by 27 per cent in mice treated with cow urine in comparison to controls. Hence in the present study also panchagavya might have enhanced the non - specific immune response which reduced the viral replication in tissues in turn minimized the exhibition of clinical signs and mortality.

In Panchagavya fed and vaccinated groups (T₆, T₇ and T₈), only two birds appeared dull, anorectic and depressed with greenish watery diarrhoea from 8 to 12 DPI in T₆ and T₇ groups. The onset of clinical signs were delayed in T₆, T₇ and T₈ as compared to T₃, T₄ and T₅ and the recovery period was also very short in T₆, T₇ and T₈ than T₂, T₃, T₄ and T₅. The intensity of clinical signs was less pronounced in T₆, T₇ and T₈ groups compared to other groups. The mortality percentage in T₆, T₇ and T₈ was 8.3 (1/12), 8.3 (1/12) and 0. Parede and Young (1990) also reported that in birds with high antibody titres (immunized birds), clinical signs are either mild or absent and there may not be any mortality after challenge with virulent field strains NDV. Dhama *et al.* (2005) reported that the cow urine one of the constituent of Panchagavya stimulated both humoral and cell mediated immune system in animals against microorganisms. In present study also the panchagavya might had a bio enhancing effect on NDV vaccine resulting in better protection, mild clinical signs and low mortality in the VNDV challenged birds.

Haemagglutination titre in VNDV challenged birds

Haemagglutination properties of Newcastle disease virus was commonly used to diagnose the disease. Haemagglutination activity is directly related to viral replication potential and load in various tissues (Alexander, 1989). The mean haemagglutination titre in pooled organ samples from dead birds was shown in Table. 1. The present study revealed that the mean HA titre was higher in vaccinated as well as vaccinated and panchgavya supplemented groups (T₂, T₆, T₇ and T₈). Even though the mean HA titre was higher in these groups, the clinical signs and mortality was lower compared to other groups. The results indicate that the vaccination protects the birds from serious consequences of disease but replication and shedding will occur when vaccinated birds challenged with virulent NDV. Previous studies also indicated that vaccination of poultry against ND can only protect birds from the more serious consequence of virulent NDV infection (severe clinical signs and mortality) but infection, replication, shedding, and transmission of virulent NDV in vaccinated birds may occur without overt disease signs (Parede and Young, 1990; Hamid *et al.*, 1991; Alexander, 2001; Kapczynski and King, 2005; Miller *et al.*, 2007).

Conclusion

The result of this study shows that the resistance against NDV was better when panchagavya was used along with immunization programme than the vaccination alone. Based on this study we recommend that in layer chicken vaccination programme should be supplemented with panchagavya for maximum protection against NDV infection.

Acknowledgement

The work described here forms part of the M.V.Sc., submitted by the first author to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The financial support provided by the Tamil Nadu Veterinary and Animal Sciences University, facilities provided by the Dean, Veterinary College and Research Institute, Namakkal are duly acknowledged.

REFERENCES

- Alexander DJ, 1989. Newcastle disease. In A laboratory manual for the isolation and identification of avian pathogens, 3rd ed, HG Purchase, LH Arp, CH Domermuth and JE Pearson (eds.). Kendall/Hunt Publishing Company, Dubuque, Iowa, pp: 114-120.
- Alexander DJ, 2001. Newcastle disease—the Gordon Memorial Lecture, British Poultry Science, 42: 5-22.
- Brugh M and CW Beard, 1980. Collection and processing of blood samples dried on paper for micro assay of Newcastle disease virus and avian influenza virus antibodies, Am J Vet Res, 41: 1495-1498.
- Bureau of Indian Standards, 1992. Nutrient requirement for poultry. 4th edn. Bureau of Indian Standards, IS. 13574: 1992, Manak Bhavan, 9, Bahadurshah Zafar Marg, New Delhi, India.
- Chakraborty D and A Chatterjee, 1998. Studies on immunomodulatory effects of levamisole in Newcastle disease vaccinated chicks, Indian J Comp Microbiol Immunol Infect Dis, 19: 85-87.
- Chauhan RS and GK Singh, 2001. Immunomodulation: An overview, Journal of Immunology and Immunopathology, 3: 1-15.
- Chauhan RS, BP Singh and LK Singhal, 2001. Immunomodulation with Kamdhenu Ark in mice, Journal of Immunology and Immunopathology, 3: 74-77.
- Dhama K, R Rathore, RS Chauhan and S Tomar, 2005. Panchagavya (Cowpathy): An overview, International journal of Cow Science, 1: 1-15.
- Gupta A, 2003. Effect of cow's urine on health of rats. MVSc, thesis submitted to College of veterinary science (Mathura), CSA University of agriculture and technology, Kanpur (Uttar Pradesh), India.
- Hamid H, RSF Campbell and L Parede, 1991. Studies of the pathology of velogenic Newcastle disease virus infection in non-immune and immune birds, Avian Pathol., 20: 561-575.
- Kapczynski DR and JD King, 2005. Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak, Vaccine, 23: 3424-3433.
- Liu XY, 1999. Stress and Immunity. In: Poultry Immunology, Yin, TB (Ed). China Agriculture press, Beijing, China, pp: 230-252.
- Mathivanan R, SC Edwin, R Amutha and K Viswanathan, 2006. Panchagavya and *Andrographis paniculata* as alternative to antibiotic growth promoter on broiler production and carcass characteristics, Int J Poultry Science, 5: 1144-1150.
- Miller PJ, DJ King, CL Afonso and DL Suarez, 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge, Vaccine, 25: 7238-7246.
- Natarajan K, 2003. Panchagavya: A Manual. Other India Press. Mapusa 403507, Goa, India.
- Parede L and PL Young, 1990. The pathogenesis of velogenic Newcastle disease virus infection of chickens of different ages and different levels of immunity. Avian Dis, 34: 803-808.
- Pazhanivel N, GA Balasubramaniam and B Mural Manohar, 2002. Pathology of Newcastle disease virus infection in immune and non-immune chicken. Indian Vet J, 79: 3-6.
- Singh, BP and RS Chauhan, 2000. Enhanced lymphocyte transformation responses in chickens due to immunoplus. XXI. World poultry congress. Montreal, Canada.
- Sumithra A, P Srinivasan and GA Balasubramaniam, 2011. Effect of Panchagavya supplementation on the performance of white leghorn cockerels. Ind J Poul Sci, 46: 124-126.