



ISSN: 2456-2912  
 VET 2019; 4(1): 19-23  
 © 2019 VET  
 www.veterinarypaper.com  
 Received: 13-11-2018  
 Accepted: 15-12-2018

**Amara Yousaf**  
 Institute of Molecular Biology  
 and Biotechnology, University of  
 Lahore, Pakistan

**MD Mehmood**  
 Ottoman Pharma (Immune  
 Division) Raiwind Road, Lahore,  
 Pakistan

**Huma Anwar**  
 Ottoman Pharma (Immune  
 Division) Raiwind Road, Lahore,  
 Pakistan

**Saud-ul-Hassan**  
 Sabroso, Model Town, Lahore,  
 Pakistan.

## Effect of Gutcare™ and Enterogermina® on humoral response of avian influenza immunization in broilers

Amara Yousaf, MD Mehmood, Huma Anwar and Saud-ul-Hassan

### Abstract

Avian influenza is highly contagious disease in commercial poultry and wild birds. The disease has been reported in broilers despite of extensive inactivated influenza virus immunization. The incriminating factors of killed vaccine failure might be sudden genetic reassortment in influenza virus or short life span of broilers. The current study is undertaken to evaluate the immunomodulatory effect of commercially available probiotics to AI immunization in broilers. Total of 16 -day old broilers were divided into four groups. Probiotics administered by two routes, in their feed and drinking water. AI vaccinated birds of Group A and Group B were offered with Gutcare™ in feed and Enterogermina® through water respectively. Whereas, group D was kept as non-vaccinated control. Blood samples collected on 20 and 40 day post vaccination were subjected for Hemagglutination Inhibition (HI) test. On 20<sup>th</sup> day post vaccination anti influenza mean HI antibody titer of group A, B, C and D was 20±8.0, 20±8.0, 12±4.62 and 0.5±1.0. On 40<sup>th</sup> day post vaccination anti influenza mean HI antibody titer of group A, B, C and D was 96±36.95, 64±0.0, 56±16 and 0±0.0. Antibody titer of Gutcare™ was statistically higher as compare to Enterogermina® on 20<sup>th</sup> and 40<sup>th</sup> day post vaccination. Gutcare™ given through feed showed significantly better effect to that of Enterogermina® offered in drinking water.

**Keywords:** Immunomodulatory effect, Gutcare™, Enterogermina®, avian influenza immunization, Hemagglutination inhibition test

### Introduction

Probiotics are non-pathogenic micro-organism used in specific amount as a supplement for both animal and human for their health benefit. Probiotics can reduce diseases by improving the immune system of host against microbial infection. Probiotics help to maintain beneficial normal flora and plays their best role to reduce the symptoms of GIT (gastrointestinal infection). It gives powerful prevention from UTI infection and diarrhea. It can give effective treatment to irritable bowel syndrome, lactose intolerance, vaginal infection, eczema and asthma in children and reduce the chances of bladder cancer [1]. Probiotics support the barrier of epithelial by regulation of genes involved in barrier function and enhance phosphorylating of adherence junction protein. Probiotics having ability to increase mucosal secretion for upgrading the barrier function. They adhere to intestinal mucus for enhancing the immune system [2]. It prevents the adhesion of pathogenic microbes like salmonella, *H. pylori*, *E. coli* and Rota virus by binding with surface protein of mucin and changes the environmental condition to make less favorable for pathogen different organic acids and bacteriosin produced by probiotics it gives stimulus to dendritic cells, epithelial cells, macrophages and lymphocytes to increase the immune response. Vast range of antibiotics of different generation used for the treatment of microbial infections but there are some undesirable effects of antibiotics on health, adverse effects on micro flora of body which leads to gastrointestinal disturbance, diarrhea and severe allergies on different body parts. Antibiotic resistance is also one of the rising issues, to overcome all these problems probiotics are good alternative of antibiotics. Live microbial fed having ability to enhance immune response [3]. So, far few strains of bacteria have been identified and being utilized in pharamaco industry for production of commercial probiotics. The most strains are streptococcus thermophilus, bacillus laterosporus, pediococcus acidilactici, bifidobactrium breve, bifidobacterium infantis, bifidobacterium bifidium, bifidobacterium lactis, bifidobacterium longum, lactobacillus bulgaricus, lactobacillus brevis, lactobacillus gasserii, lactobacillus casei, lactobacillus

**Correspondence**  
**MD Mehmood**  
 Ottoman Pharma (Immune  
 Division) Raiwind Road, Lahore,  
 Pakistan

plantrum, lactobacillus lactis and lactobacillus rehamnosus [4]. However, with the advancement in science and technology, many new types are being explored with much better response. The research has been carried out on some other species named as clostridium butyricum, bifidobacterium (Gutcare™) and bacillus clausii (Enterogermina®)

**Material and Methodology**

**Source of chicks**

24-day old broiler chicks were purchased from Big Bird Poultry Breeders Lahore, Pakistan. The birds were shifted to clean and disinfected experimental house of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore as shown in Fig. 1.



**Fig 1:** Brooding and rearing of experimental broilers

**Source of vaccine and antigen**

300 ml of vaccine two ml of inactivated antigens were purchased from the Ottoman Pharma (Immuno Division) Lahore, Pakistan. The vaccine and antigen were transported in cold chain and kept at in refrigerator (4°C) located Biotechnology and Vaccinology Laboratory, IMBB till further use.

**Evaluation of antigen**

HA titer of the antigen was determined by the method as describe by Seidavi [5].



**Fig 2:** 4HA determination by Heamagglutination assay

**Source of probiotics**

Probiotic sachet of 500 mg Gutcare™ and oral suspension of Enterogermina® (2bi/5mL) manufactured by Shandong Kexing Bioproducts Co., Ltd. CRC and sanofi-aventis S. p. A –Viale Europa, 11-Origgio (VA)-ITALY respectively were

purchased from the Pharmacy of Teaching Hospital of The University Of Lahore as depicted in Fig.3.



**Fig 3:** Experimental probiotics

**Experimental design**

Total of 16 birds were divided into four groups on the basis of different colors A, B, C & D each containing 4 birds were kept in separate cages. All the birds in group A, B and C were vaccinated at day 7 with 0.3 mL/dose of OTTO FLU VAC subcutaneously. Each bird of the group A and B were offered with 2gm/liter of Gutcare™ and Enterogermina® respectively on 5<sup>th</sup> day of age for 10 days consecutively. The broilers in Group C were immunized with OTTO FLU VAC without been probiotic supplementation whereas, birds in group D were kept as unvaccinated non probiotic served control as shown in Fig. 4.



**Fig 4:** Vaccination and Group classification

**Collection of blood sample**

One ml of blood was collected in three ml of disposable syringe from the wing vein of each bird of every group on 20<sup>th</sup> and 40<sup>th</sup> day post vaccination as shown in Fig. 5.



**Fig 5:** Blood sampling

**Extraction of serum**

The syringes containing blood were kept at slant position and room temperature for overnight to separate. The serum thus separated was stored at -60 °C till further use.

**Seroconversion through HI assay**

50 ul of normal saline was dispensed in 1-12<sup>th</sup> well of 96 well microtiter plates. 50 ul of extracted serum was added in 1<sup>st</sup> well and made 2 fold dilutions up to 10<sup>th</sup> well. After that 50 ul of Ag (4HA) was dispensed from 1<sup>st</sup> to the 11<sup>th</sup> well and incubated it at 37 °C for 20 mins. Similarly 50 ul of 1% washed chicken RBCs were added up to the 12<sup>th</sup> well and incubated for 20 mins.

**Statistical analysis**

Results were analyzed through mean standard deviation and subsequently by bonferroni. P. value <0.05 showed significant results.

**Results**

Avian influenza inactivated vaccine inoculated birds offered with Gutcare™ in feed and Enterogermina® through water separately induced detectable anti AIHI-antibody titers on 20 and 40 days post vaccination.

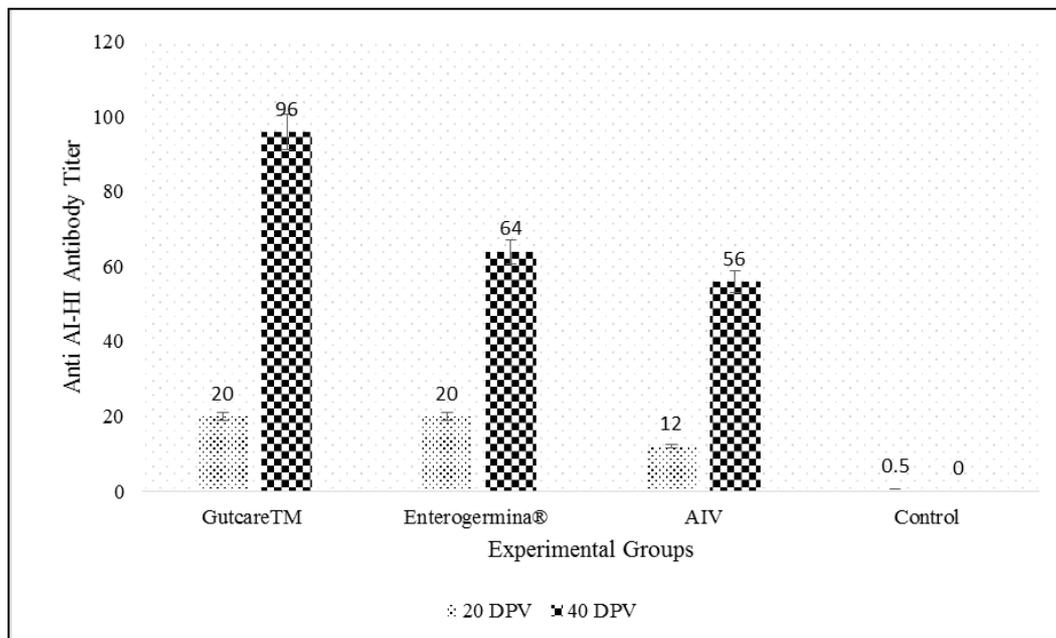
- On 20<sup>th</sup> day post vaccination bird 1, 2, 3 and 4 offered with Gutcare™ treated feed of group A showed 16, 16, 16 and 32 anti-AIHI antibody titer respectively. The mean anti AIHI antibody titer was 20. Whereas, On 40<sup>th</sup>

day post vaccination bird 1, 2, 3 and 4 offered with Gutcare™ treated feed of group A showed 128, 128, 64 and 64 anti-AIHI antibody titer respectively. The mean anti AIHI antibody titer was 96.

- On 20<sup>th</sup> day post vaccination bird 1, 2, 3 and 4 offered with Enterogermina® treated water of group B showed 32, 16, 16 and 16 anti-AIHI antibody titer respectively. The mean anti AIHI antibody titer was 20. Whereas, on 40<sup>th</sup> day post vaccination bird 1, 2, 3 and 4 offered with Enterogermina® treated water of group B showed 64, 64, 64 and 64 anti AIHI antibody titer respectively. The mean anti-AIHI antibody titer was 64.
- On 20<sup>th</sup> day post vaccination bird 1, 2, 3 and 4 offered with simple feed of group C showed 16, 16, 08 and 08 anti-AIHI antibody titer respectively. The mean anti AIHI antibody titer was 12. Whereas, On 40<sup>th</sup> day post vaccination bird 1, 2, 3 and 4 offered with simple feed of group C showed 32, 64, 64 and 64 anti-AIHI antibody titer respectively. The mean anti AIHI antibody titer was 56.
- On 20<sup>th</sup> day bird 1, 2, 3 and 4 offered with simple feed of control group D showed 2, 0, 0 and 0 anti AIHI antibody titer respectively. The mean anti-AIHI antibody titer was 0.5. Whereas, on 40<sup>th</sup> day bird 1, 2, 3 and 4 offered with simple feed of group D showed 0, 0, 0 and 0 anti-AIHI antibody titer respectively. The mean anti AIHI antibody titer was 0. 4HA determined in well # 8, 256 HA unit.

**Table 1:** Effect of Gutcare™, Enterogermina® and avian influenza oil based vaccine on humoral response of AI immunization in broilers on 20 and 40 DPV.

Sr.	Groups of birds	Anti-influenza Antibody Titer 20 day PV	Anti-influenza Antibody Titer 40 day PV
1.	Gutcare™ treated feed	16, 16, 32, 16=20±8.0	128, 128, 64, 64=96±36.95
2.	Enterogermina® treated water	32, 16, 16, 16=20±8.0	64, 64, 64, 64=64±0.0
3.	AI vaccinated	16, 16, 08, 08=12±4.62	32, 64, 64, 64=56±16
4.	Control	02, 0, 0, 0=0.5±1.0	0, 0, 0, 0=0±0.0



**Fig 6:** Effect of Gutcare™, Enterogermina® water and avian influenza oil based vaccine on humoral response of AI immunization in broilers on 20 and 40 DPV.

- Gutcare™: Probiotic Feed Treatment
- Enterogermina®: Probiotic Water Treatment
- AIV: Avian Influenza Vaccine

**Discussion**

Probiotics are included in the group of biologicals used as preventive measures to control the severity of disease. It is basically composed of single or multiple strains of non-

pathogenic microorganisms in freeze dried forms which reactivate inside the body. Such organisms have the potential to grow much faster in limited period of time and prevent the host from the wild virulent strains by competing the nutritional requirements, encapsulating the host receptors and provision of essential amino acids and vitamins. The immune system of the living organisms is complex process augmented by various physiochemical factors. Currently the probiotics are being extensively used in humans and poultry to overcome the infectious diseases. The current study was designed to evaluate the effect of probiotics in vaccinated chicken. For the purpose Gutcare™ and Enterogermina® were selected and offered to the vaccinated healthy birds through feed and drinking water respectively. The broilers divided into four different groups were kept under similar environmental conditions.

It is observed that Gutcare™ provided in feed to the vaccinated broilers showed  $20 \pm 8$  and  $96 \pm 36.95$  mean anti H9-HI antibody titer at 20<sup>th</sup> day and 40<sup>th</sup> day post vaccination respectively (Table-1, Fig. 6). The antibodies titers are significantly raised at 40<sup>th</sup> day as compare to the 20 day ( $p < 0.05$ ). Our results are in accordance with the results of Abeer who reported that protexin augmented the effect of inactivated influenza vaccine [6]. The results of the current study are also in lines with the observation of Manafi who declared that microguard has the enormous effect on immunogenesis in vaccinated flocks against influenza and Newcastle disease vaccine [7]. Nayebpor conducted similar study and reported satisfactory results of primalac on broiler immunity, physical fitness and on intestinal micro flora [8]. Another author Ghafoor revealed that use of multistrain probiotics in feed enhance the immunomodulatory effect of oil based avian influenza vaccine [9].

It is observed that Enterogermina® provided in drinking water to the vaccinated broilers showed  $20 \pm 8$  and  $64 \pm 0.0$  mean anti H9-HI antibody titer at 20 days and 40 day post vaccination respectively (Table-1, Fig. 6). The antibodies titers are significantly raised at 40<sup>th</sup> day as compare to the 20 day ( $p < 0.05$ ). The results of Torshizi experiment also supported the positive effect of protexin on humoral response and growth [10]. In contrast Talazadeh evaluated non-significant results of bifidobacterium, lactobacillus and streptococcus on immune response of avian influenza vaccine [11]. Whereas Zhang demonstrate that bioplus2b had beneficial effects on immunity and egg quality [12].

The role of artificial active immunization in birds is highly critical and its in-vivo humoral response could be evaluated by using different techniques. The use of vaccines was specifically recommended in healthy host. The reason is that normally immunogenesis required three major steps for the optimal response. 1). Good processing of antigen to the antigen processing cells (APC) inside the body. 2). Presentation of processed antigen to the Helper T cells. 3). Optimal activation of Plasma cells and cytotoxic T cells. All the stages of immunogenesis required active and sufficient number of immune cells to induct immediate and effective immune response. Whenever, foreign invaders attack the host/immune system it becomes vulnerable to the destruction in terms of minimal antigen processing, presentation and weak coordination between the immunogenic cells. In such conditions probiotics play a vital role to rehabilitate the structural and functional organization of immune system. Probiotics enhances the uptake and phosphorylating of adherence junction genes resulting maximum adherence to the intestinal mucosa which prevents the attachment of

pathogenic bacteria like [2]. Due to the rapid growth of probiotic bacteria it creates nutritional deficiencies for the opportunistic and pathogenic bacteria. Probio strains can interact with macrophages, dendritic cells, lymphocytes and epithelial cells. Interstitial epithelial cells of host have ability to interact with probiotics. Dendritic cells interacting with probiotics can trigger innate and acquired immunity. In case of innate immunity PAMPs (pathogen associated molecular patterns) are involved, pattern recognition receptor (PRRs) bind with PAMPs and generated primary response. Mostly toll like receptors behaved as PRRs. Whereas NLRs and CLRs are signal transmitters which transmit signal after binding with bacteria. In contrast B and T lymphocytes are involved in acquired immune response.

### Conclusion

The current study revealed that Gutcare™ and Enterogermina® have better immunomodulatory effect of avian influenza vaccine in broiler chicken. Gutcare™ given through feed has significant better effect rather than Enterogermina® offered in drinking water. So, it's recommended that Gutcare™ administered by the route of feed will enhance the humoral response against Avian Influenza Virus. It is therefore, concluded that probiotics may play a vital role in the augmentation of immune response in association with inactivated influenza vaccines where antigen processing and presentation is always been the question of mark.

### Acknowledgement

The author is highly grateful to the Rauf Khalid CEO Ottoman Pharma, Lahore, Pakistan for providing financial support. I am also thankful to our colleagues Muhammad Ismael, Usman Ghani and Dr. Sajjad Hussain who offered technical support and assisted the research.

### References

1. Michael T, Murray ND. is a director of product development and education in natural health and co-author of The Encyclopedia of Natural Medicine.
2. Goubeyre P, Denery S, Bodinier M. Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. *Journal of leukocyte biology*. 2011; 89(5):685-695.
3. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. *The American journal of gastroenterology*. 2006; 101(4):812.
4. Collins JK, Thornton G, Sullivan GO. Selection of probiotic strains for human applications. *International dairy journal*. 1998; 8(5, 6):487-490.
5. Seidavi A, Dadashbeiki M, Alimohammadi-Saraei MH, van den Hoven R, Payan-Carreira R, Laudadio V, *et al*. Effects of dietary inclusion level of a mixture of probiotic cultures and enzymes on broiler chickens immunity response. *Environmental Science and Pollution Research*. 2017; 24(5):4637-4644.
6. Abeer SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY *et al*. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry science*. 2013; 92(3):663-670.
7. Manafi M, Hedayati M, Mirzaie S. Probiotic *Bacillus* species and *Saccharomyces boulardii* improve performance, gut histology and immunity in broiler

- chickens. South African Journal of Animal Science. 2018; 48(2)
8. Nayebpor M, Farhomand P, Hashemi A. Effects of different levels of direct fed microbial (Primalac) on growth performance and humoral immune response in broiler chickens. J Anim. Vet. Adv. 2007; 6(11):1308-1313.
  9. Ghafoor A, Naseem S, Younus M, Nazir J. Immunomodulatory effects of multistrain probiotics (Protexin™) on broiler chicken vaccinated against avian influenza virus (H9). International Journal of Poultry Science. 2005; 4(10):777-780.
  10. Torshizi KM, Moghaddam A, Rahimi S, Mojangani N. Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. Br. Poult. Sci. 2010; 51:178-184.
  11. Talazadeh F, Mayahi M, Zeinali S. The effect of Aquablend Avian probiotic® including Lactobacillus, Streptococcus and Bifidobacterium on systemic antibody response against Newcastle and Influenza disease vaccine in broiler chickens. Int J Enteric Pathog. 2016; 4(2):1-5.
  12. Zhang JL, Xie QM, Ji J, Yang WH, Wu YB, Li C *et al.* Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens. Poultry Science. 2012; 91(11): 2755-2760.