Clinical Evaluation the efficacy of Tri-Reo vaccine in broiler breeders by ELISA method

Sina Sedghi[1], Adel Feizi[2*], Saied Mokhtari[1]

1. Students of Veterinary Medicine, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

* Corresponding Author: adel_feizi@yahoo.com

Abstract: Reoviruses are ubiquitous in chickens and turkeys; some strains become viremic and localize in the large joints, resulting in arthritis, tendinitis, and synovitis. Avian Reovirus Vaccine is a Killed Virus for the subcutaneous or intramuscular revaccination of healthy chickens 10 weeks of age as an aid in the prevention of signs and lesions associated with avian reovirus infections which cause malabsorption syndrome. The aim of present study was to clinical evaluation the efficacy of Tri-Reo vaccine in broiler breeders by ELISA method. In this study which carried out during the 2012, we worked on the poultries of 2 farms with 6 halls in each, in that, salon No. 6 considered as control group. Others were considered as experimental group with different titrs of vaccine. In these farms, the first Tri-Reo vaccination applied on week 7 and the second time was on week 19. In fact, the Tri-Reo vaccine was administrated two times during the study. At the end, blood samples were obtained from chickens for laboratory procedures. The data showed that the titr obtained on day 1 was lesser than titr obtained after second vaccination in both farms ($p<0.05$). In conclusion, it shows the necessity of further investigations in other parts of our country as well as other flocks such as layers, layer breeders and broilers. The investigations should consider different aspects of the disease to identify risk factors which may be responsible for pathogenicity of the virus.

[Sina Sedghi, Adel Feizi, Saied Mokhtari. **Clinical Evaluation the efficacy of Tri-Reo vaccine in broiler breeders by ELISA method.** *Life Sci J* 2013;10(4s):423-426] (ISSN: 1097-8135). http://www.lifesciencesite.com. 64

Keywords: Tri-Reo, Reovirus, vaccine, broiler breeder, ELISA.

1. Introduction

Reoviruses are ubiquitous in chickens and turkeys; some strains become viremic and localize in the large joints, resulting in arthritis, tendinitis, and synovitis. Most birds are thought to be susceptible to respiratory-intestinal strains of reoviruses (Krauss and Ueberschar, 1966). Chickens and, to a lesser degree, turkeys are susceptible to viral arthritis, which is seen worldwide. Reoviruses also have been associated with pericarditis and myocarditis, hydropericardium, pasting, malabsorption, and femoral head necrosis, although further study is needed to define their role (Dutta and Pomeroy, 1969).

The disease is egg-transmitted and is of short duration except when lateral transmission in a flock is prolonged. Respiratory and digestive infections may occur but are of short duration; however, the virus survives in tendon sheaths for extended periods. The virus is spread via aerosols, fomites, and mechanical means, and is resistant to heat and chemical inactivation (Dutta and Pomeroy, 1969).

Several antigenic subtypes of avian reoviruses have been identified; however, there appears to be significant cross-protection among most of the isolates or subtypes. Pathogenicity of the isolates varies widely. Serious outbreaks of viral arthritis are followed by a decreased incidence in later hatch groups of birds from the same parent flock (Fahey and Crawley, 1954; Petek et al., 1967). This may be related to decreased egg transmission and development of parental immunity. Day-old chicks are more susceptible than older birds when exposed by natural means. The earlier in life the chick is infected, the longer the virus persists in the tissues (Bains et al., 1974).

The arthritic form (tenosynovitis) usually is seen in broilers 4-8 wk old as unilateral or bilateral swellings of the tendons of the shank and above the hock; it can also be found in much older chickens. The birds walk with a stilted gait. In severely affected flocks, rupture of the gastrocnemius tendon is frequent, and many cull birds are seen around the feeders and waterers. Mortality is 2-10% and morbidity 5-50%. Severely affected birds rarely recover; less severely affected birds recover in 4-6 wk. The infection is inapparent in many birds. Feed efficiency and rate of gain are decreased (Goodwin et al., 1993; Page et al., 1982).

An acute, fulminating infection is occasionally seen in young chicks and embryos with cardiomegaly, hepatomegaly, and splenomegaly with necrotic foci. Edema of the tendons of the leg is marked, petechial hemorrhages develop in the synovial membranes above the hock, and fusion and calcification of the tendon bundles are common.

Blood clots and hemorrhages are seen with rupture of the gastrocnemius tendon. Pitted erosions of the cartilage of the distal tibiotarsus are seen with flattening of the condyles (Pass et al., 1982; Van der Heide et al., 1981). Histologically, the synovial cells are hypertrophied, hyperplastic, and infiltrated by lymphocytes and macrophages. The synovia contains heterophils and macrophages. Infiltration of heterophils or lymphocytes, or both, between myocardial fibers is a constant finding. However, the infiltrating heterophils are difficult to distinguish from the clusters of young, proliferating heterophils (ectopic myelopoiesis) that are present in the heart muscle of all young, rapidly growing broiler chickens (Vertommen et al., 1980; McFerran et al., 1976). A presumptive diagnosis can be based on unilateral or bilateral swelling of the tendons of the shank and tendon bundle above the hock and on the inflammatory changes in the tendons and synovia described above. Virus from affected tissues can be isolated in primary kidney, liver, or lung cells, or in the yolk sac or chorioallantoic membrane of embryonating chicken eggs. The agar-gel-precipitin test is usually positive, and most birds are positive early in the infection. Virus neutralization tests and challenge of immunized chickens are used to detect the specific serotype. Culture procedures should be used to differentiate mycoplasmal and other bacterial infections. Other causes of lameness should be considered (Bagust and Westbury, 1975).

Avian Reovirus Vaccine is a Killed Virus for the subcutaneous or intramuscular revaccination of healthy chickens 10 weeks of age as an aid in the prevention of signs and lesions associated with avian reovirus infections which cause malabsorption syndrome. Progeny of vaccinates are aided in the prevention of signs of malabsorption associated with reovirus disease via maternal antibodies. It is essential for best protection to prime birds at least once with live virus vaccine for Tenosynovitis (Cook, 1991). The aim of present study was to clinical evaluation the efficacy of Tri-Reo vaccine in broiler breeders by ELISA method.

2. Materials and methods

In this study which carried out during the 2012, we worked on the poultries of 2 farms with 6 halls in each, in that, salon No. 6 considered as control group. Others were considered as experimental group with different titrs of vaccine. The total number of poultries was 97000 broiler breeders. In these farms, the first Tri-Reo vaccination applied on week 7 and the second time was on week 19. In fact, the Tri-Reo vaccine was administrated two times during the study. The other vaccines that chickens received were as below:

Bronchitis vaccine (H120), levack vaccine, dual live vaccine Newcastle+bronchitis, live bronchitis vaccine (4/91), dual killed vaccine clone+influenza, LaSota, Gambro live vaccine, MS live vaccine, ORT killed vaccine, TRT killed vaccine, CAV, POX, ILT, infectious coryza, AE vaccine and quad vaccine. It must be noted that chickens have not received Tri-Reo vaccine so far. At the end of the period, blood samples were obtained by chance and were analyzed in term of Tri-Reo vaccine titr using the IDEXX ELISA kit. The data were compared with those obtained from the day 1 analyses.

Data were analyzed statistically using the SPSS software and $P<0.05$ considered as statistically significant.

3. Results

In general, the data showed that the titr obtained on day 1 was lesser than titr obtained after second vaccination in both farms ($p<0.05$). In farm No. 1, the mean value of titr prior the study and after second vaccination was 7500.8 and 19754.6, respectively (table 1). In farm No. 2, the mean value of titr prior the study and after second vaccination was 9161.667 and 17379.17, respectively (table 2).

Table 1: titrs obtained from chickens of farm No. 1

No. of hall	Titr on day 1 (CV%)	Titr before first vaccination (CV%)	Titr after second vaccination (CV%)
1	10135 (20.1)	1036 (60.4)	22738 (27)
2	6068 (44.8)	470 (31.1)	23671 (31)
3	9097 (17.1)	1241 (47.8)	23289 (20)
4	6721 (22.2)	351 (38.6)	22670 (28)
5	4627 (37)	888 (61.1)	25276 (33)
6	8357 (19)	884 (44)	884 (44)

Table 2: titrs obtained from chickens of farm No. 2

No. of hall	Titr on day 1 (CV%)	Titr before first vaccination (CV%)	Titr after second vaccination (CV%)
1	9836 (59.4)	5322.7 (8)	17727 (25)
2	9050 (58.3)	5403.4 (9.1)	16217 (23.5)
3	9624 (57.8)	5356.6 (8.9)	17439 (29.7)
4	8989 (54.6)	5402.3 (7.1)	18545 (17.5)
5	9015 (57.4)	5423.3 (7.8)	17797 (18.7)
6	8456 (56.3)	5381 (9)	16550 (18.5)

4. Discussion and conclusion

Vaccination plays an important part in the health management of the poultry flock. There are numerous diseases that are prevented by vaccinating the birds against them (Bacon, 1992; Keck et al., 1993). The purpose in using a vaccine to prevent a particular disease is to trigger or boost the bird's immune system to produce antibodies that in turn

fight the invading causal organisms. A natural invasion that actually causes the disease will have the same result – the bird will produce antibodies that fight future invasion. Unfortunately the damage done to the bird suffering such disease is usually too great and the bird either dies or becomes unthrifty and non-productive. A natural invasion caused infection will be uncontrolled and has the possibility of causing severe damage. Vaccination is a way of obtaining a controlled result with a minimum of harm to the birds. Vaccines are generally fragile products some of which are live but in a state of suspended animation (Cook, 1991). Others are dead. All have a finite life that is governed by the way they are handled and used. Handling and administration procedures also influence the potency of many vaccines and consequently the level of immunity the bird develops. After administering a live vaccine in poultry, the vaccine virus must infect target cells and replicate, increasing their numbers in order to stimulate the immune system (Baxendale, 1996). If the vaccine is administered properly to healthy birds, a 'normal' vaccine reaction will occur (Baxendale, 1996).

Avian reoviruses have also been associated with other disease conditions in chickens where the role of the virus is less clear and indeed sometimes tenuous. These include enteric problems such as cloacal pasting and mortality (Dutta and Pomeroy, 1969), ulcerative enteritis (Krauss and Ueberschar, 1966), enteric disease (Dutta and Pomeroy, 1969), respiratory disease (Fahey and Crawley, 1954; Petek et al., 1967), inclusion body hepatitis (McFerran et al., 1976), increased mortality and heart lesions in young broilers (Bains et al., 1974), sudden deaths in young broilers associated with lesions in the heart, kidney and liver (Bagust and Westbury, 1975) and the variously named runting/malabsorption/brittle bone disease in young broilers (Goodwin et al., 1993; Page et al., 1982; Pass et al., 1982; Van der Heide et al., 1981; Vertommen et al., 1980). Recently, sudden deaths have been reported in young broilers in Poland. The disease was characterised by liver lesions, from which a reovirus was isolated which could reproduce the disease experimentally (Z. Minta, personal communication).

Avian Reovirus has been implicated in many disease syndromes and is not discernible from other poultry diseases by clinical examination; therefore laboratory diagnosis of the disease is required. In comparison with the existing antibody assay technique in viral neutralization of AGP, the ELISA method offers high sensitivity and is more simple, faster and less expensive (Slaght *et al*., 1978). But this test is not effective to detect all Reovirus strains and serotypes. So negative results obtained by commercial ELISA cannot reject the presence of anti-Reovirus antibodies. This fact restricts the results for interpretation. Furthermore, ELISA test cannot distinguish between Reovirus vaccine and natural Reovirus antibodies. Reovirus-associated disease has been reported predominantly in the United States (Glass *et al*., 1973; Dobson and Glisson, 1992; De Herdt *et al*., 1999). In Europe clinical signs of the disease have been observed sporadically (De Herdt *et al*., 1999). Results derived from a seroprevalence study on Nigerian poultry show that the prevalence of Reovirus antibody is 41% (Owoade *et al*., 2006). In Iran for the first time Khodashenas and Aghakhan (1992) isolated and characterized avian Reovirus from the case with malabsorption syndrome and arthritis/tenosynovitis. In conclusion considering the high prevalence put emphasis on the vaccination of the breeder flocks to reduce the economic losses of the disease. It also shows the necessity of further investigations in other parts of our country as well as other flocks such as layers, layer breeders and broilers. The investigations should consider different aspects of the disease to identify risk factors which may be responsible for pathogenicity of the virus.

References:

1. Bacon LD. Measurement of immune competence in chickens. Poult Sci Rev 1992;4:187–195.
2. Bagust TJ, Westbury HA. Isolation of reoviruses associated with diseases of chickens in Victoria. Aust vet 1975;51:406-407.
3. Bains BS, Mackenzie M, Spradbrow PB. Reoviruses associated with mortality in broiler chickens. Avian Dis 1974;18:472-476.
4. Baxendale W. Current methods of delivery of poultry vaccines. In: Poultry Immunology. Davison TF, Morris TR, Payne LN, (Eds.). Carfax Publishing Company, Oxfordshire 1996;375–387.
5. Cook ME. Nutrition and the immune response of domestic fowl. Crit Rev Poult Biol 1991;3:167–189.
6. De Herdt P, Ducatelle R, Uyttebroek E, Hermans J, Sneep A, Torbeyns R. Reovirus serology in broiler parents and their progeny and its correlation with performance. Avian Dis 1999;43:271-278.
7. Dobson KN, Glisson JR. Impact of a documented case of Reovirus infection in broiler breeders. Avian Dis 1992;36:788-791.
8. Dutta SK, Pomeroy BS. Isolation and characterisation of an enterovirus from baby chicks having an enteric infection. II. Physical and chemical characterisation and ultrastructure. Avian Dis 1969;11:9-15.

9. Fahey JE, Crawley JF. Studies on chronic respiratory diseases of chickens. II: Isolation of a vims. Can J comp Med 1954;18:13-21.
10. Glass SE, Naqi SA, Hall CF, Kerr KM. Isolation and characterization of a virus associated with arthritis of chickens. Avian Dis 1973;17:415-424.
11. Goodwin MA, Davis JF, McNulty MS, Brown J, Player EC. Enteritis (so-called runting stunting syndrome) in Georgia broiler chicks. Avian Dis 1993;37,451-458.
12. Keck LD, Skeeles JK, McNew RW. Antibody detection in matched chicken sera and egg-yolk samples by commercial enzyme-linked immunosorbant assay kits for Newcastle disease virus, infectious bronchitis virus, infectious bursal disease virus, and avian reovirus. Avian Dis 1993;37:825–828.
13. Khodashenas M, Aghakhan SM. Isolation and characterization of avian Reoviruses from the cases of malabsorption syndrome and arthritis/tenosynovitis in chickens. Arch Inst Razi 1992;42/43:103-116.
14. Krauss H, Ueberschar S. Zur Structur eines neuen Geflügel-Orphanvirus. Zentralbl Veterinärmed 1966;13:239-249.
15. McFerran JB, McCracken RM, Connor TJ, Evans RT. Isolation of viruses from clinical outbreaks of inclusion body hepatitis. Avian Pathol 1976;5:315-324.
16. Owoade AA, Ducatez MF, Muller CP. Seroprevalence of avian influenza virus, infectious bronchitis virus, Reovirus, avian Pneumovirus, infectious laryngotracheitis virus, and avian leucosis virus in Nigerian poultry. Avian Dis 2006;50:222-227.
17. Page RK, Fletcher OJ, Rowland GN, Gaudry D, Villegas P. Malabsorption syndrome in broiler chickens. Avian Dis 1982;26:618-624.
18. Pass DA, Robertson DM, Wilcox GE. Runting syndrome in broiler chickens in Australia. Vet Ree 1982;110:386-387.
19. Petek M, Feiluga B, Borghi G, Baroni A. The crawley agent: an avian reovirus. Arch ges Virusforsch 1967;21:413-424.
20. Slaght SS, Yang TJ, Van der Heide L, Fredrickson TN. An enzyme-linked immunosorbent assay (ELISA) for detecting chicken anti-reovirus antibody at high sensitivity. Avian Dis 1978;22:802-805.
21. Van der Heide L, Lutticken D, Horzinek M. Isolation of avian reovirus as a possible etiologic agent of osteoporosis ('brittle bone disease', 'femoral head necrosis') in broiler chickens. Avian Dis 1981;25:847-856.
22. Vertommen M, van Eck JHH, Kouwenhoven B, van Nol K. Infectious stunting and leg weakness in broilers: I. Pathology and biochemical changes in blood plasma. Avian Pathol 1980;9:133-142.

2/14/2013