Cross-protection of the Reo2 GC5 Live Vaccine Against Challenge with a Virulent Reovirus GC2

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ABSTRACT

The introduction of inactivated Reovirus 2 vaccines (GC5) as part of the vaccination protocol in breeding flocks in 2016 in Israel, did not prevent infection and shedding of the virus to the progeny. In 2019 a live embryo adapted non-attenuated Reovirus 2 (GC5) live vaccine, was approved by the Israeli Veterinary Services to be introduced as part of the vaccination program against Reovirus infection in broiler breeders' replacement pullets under controlled and monitored conditions as a controlled exposure approach. Based on the epidemiological data, it was found that the prevalence of other Reoviruses, such as group 3 (GC2), that were present in the past, was reduced after the introduction of the Reovirus group 2 (GC5) live vaccine. The purpose of this study was to test the ability of the Reovirus 2 live vaccine to provide protection against a challenge with a different virulent genotype of the Reovirus (Reovirus C3-GC2) under controlled conditions in SPF birds.

Keywords: ARV – Avian Reovirus; GC – Genotype Clusters; SPF – Specific Pathogen Free.

INTRODUCTION

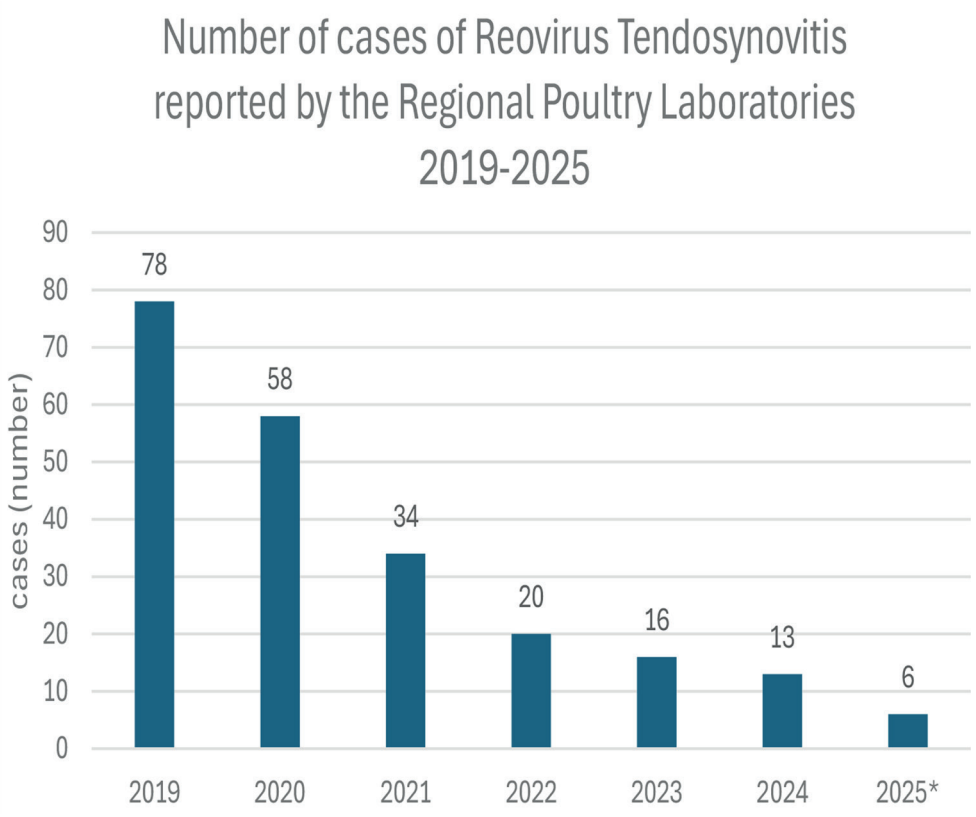
In the last decades, Reovirus tendosynovitis became one of the most important challenges for the poultry industry in many countries (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). Reovirus is the most important causal agent of arthritis/tenosynovitis in chickens, especially in heavy breeds. Early infection with Reovirus either by vertical or horizontal transmission, causes inflammation and scarring of the gastrocnemius and flexor tendons, leading to lameness, tendon rupture, and abnormal leg spreading leading to economic losses and critical welfare issues (1, 2, 3, 4, 5, 6, 9, 10). In Israel, the economic impact of the disease is higher due to the significant condemnation rates related to Jewish Kosher laws (1, 10, 11).

Genetic diversity among ARV strains occurs through segment reassortment and mutations in the viral genome, mainly the S1 segment encoding the Sigma C (σC) protein that is responsible for its attachment to the cell receptors and induction of specific neutralizing antibodies (13, 14, 15, 16, 17, 18). In most countries, the control of the Reovirus outbreaks is based on the development of autogenous inactivated vaccines. Due to the appearance of variant strains with different antigenic characteristics, it is necessary to vaccinate with the specific prevalent genotype of Reovirus to induce an effective immune response and protection (3, 15).

It has been suggested that the use of specific autogenous inactivated vaccine may favor and increase the development and spreading of other genetic clusters of Reovirus present in the area (13, 16).

During the last two decades, severe outbreaks of Reovirus tendosynovitis caused by different Genotype Clusters were observed in Israel and many other countries (1, 2, 7, 8, 13, 14).

According to the epidemiological data obtained from the Israeli Veterinary Services and the Regional Poultry Diagnostic Laboratories, different genotype clusters (GC)



* From January to May 2025.

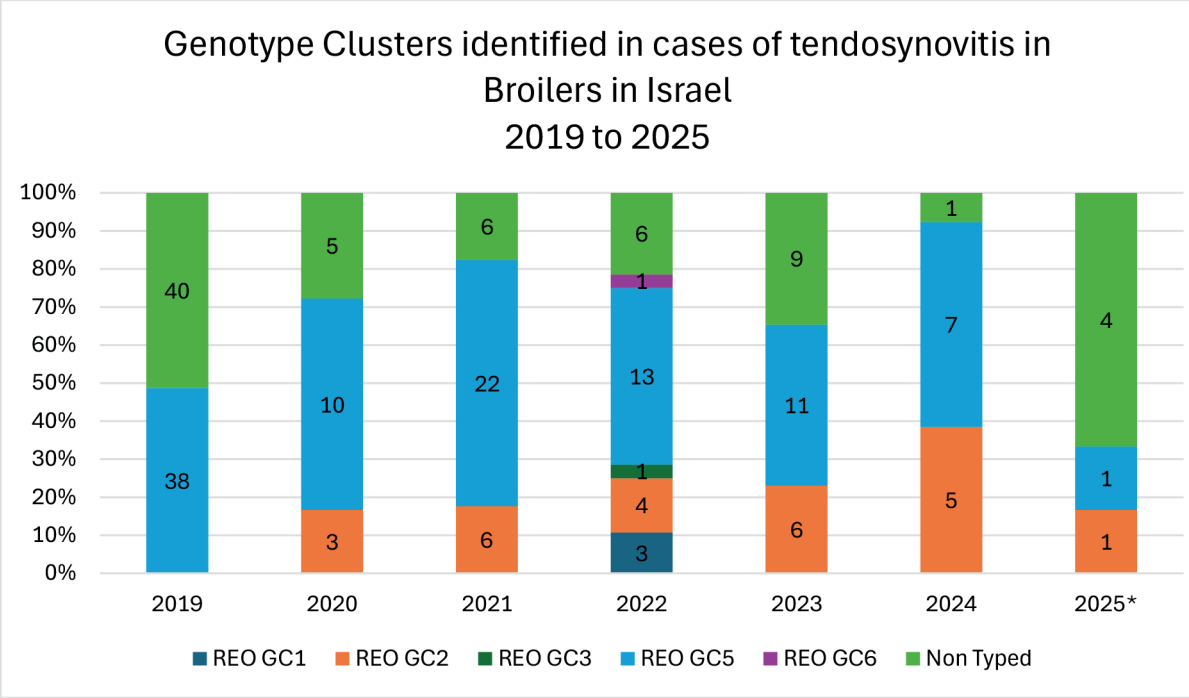
Figure 1. Number of cases of Tendosynovitis reported by the Regional Poultry Diagnostic Laboratories from 2019 to 2025.

of Reovirus have been involved in cases of tendosynovitis in breeders and broilers in Israel. Two Genotype clusters of Reovirus, GC5 (previously defined as variant 2) and GC2, have been the most prevalent genotype clusters, with GC5 (previously defined as variant 3) causing most of the outbreaks of tendosynovitis in Israel in the last decades. In the last years, GC2 Reovirus was involved in several cases of tendosynovitis in broilers causing increased condemnation rates in the slaughterhouses (1,10, 13).

Since the introduction of the Reo2 live vaccine (Abic Biological Laboratories, Israel) in 2019, outbreaks of Reovirus tendosynovitis caused by the GC5 virus, decreased by more than 90% (Fig. 1), but interestingly at the same time the number of cases of tendosynovitis caused by the GC2 did not increase (Fig. 2) as observed in countries using the autogenous inactivated vaccines of different genotype clones (3, 13, 21), suggesting a possible cross protection of the Reo2 live vaccine against the GC2 Reovirus. To test the theoretical assumption of cross protection provided by the Reo2 live vaccine, a controlled study was carried out in SPF chicks based on vaccination and challenge by the foot-pad inoculation and lesion scoring (19, 20, 21).

MATERIALS AND METHODS

1. **Animals**: Sixty, one-day old SPF chicks from the same origin and hatch, were randomly separated into five groups of 12 chicks and each group was allocated to an isolator unit at the Herut experimental farm (Abic Biological Laboratories, Israel). All the chicks received commercial crumbled feed and water *ad libitum* and were maintained under recommended management conditions of light, temperature and ventilation.
   Daily monitoring and recording were carried out to determine the health of the birds.
2. **Vaccine**: The Reo2 live vaccine (GC5), batch No:21131005 (Abic Biological Laboratories Ltd., Israel), was used for vaccination of the chicks, the vaccine was kept frozen until the time of use, thawed and diluted in PBS solution to obtain one dose / bird containing $10^{3.5}$ EID_{50}/dose of 0.5ml.



* From January to May 2025

Figure 2. Reovirus genotypic clusters (GC) identified in Israel from 2019 to 2025 as reported by the Regional Poultry Diagnostic Laboratories in Israel.

Table 1: Description and classification by GC of the challenge viruses

Actual Genotype Cluster (GC)	Previous Classification	Isolate #	Titer of stock (EID50/ml)	Challenge dose & volume/dose
GC5	Group 2	7585	10^6.5 EID_{50}/ml	10^3.0 EID_{50}/dose (0.05ml/dose)
GC2	Group 3	400356	10^7.2 EID_{50}/ml	

3. **Challenge Viruses**: The viruses were obtained from the Regional Poultry Diagnostic Laboratories in Israel. The challenge was carried out using the two most prevalent Reoviruses in Israel (Reovirus GC5 and Reovirus GC3). The Isolation and classification were carried out by the Israel Veterinary Institute in Beith Dagan by sequencing the sigma C as previously described (10) and supplied to Abic Biological Laboratories Ltd. for research and vaccines production.

   3.1 Titration of Reovirus in embryonated eggs: The challenge viruses were diluted in sterile PBS at 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} and injected into the yolk of SPF embryonated eggs for the virus titration. Six SPF embryonated eggs were used per each dilution for each virus. The embryos were examined daily for 7 days for mortality and pathological signs. The titer was calculated using the Reed and Munch formulation to obtain the Embryo Infective Dose 50% (EID_{50}) as summarized in Table 1.

4. **Vaccination and challenge Program**: The SPF chicks were reared in the isolator units with no other vaccine or treatment. At the age of 6 weeks, the birds in Group 1 and Group 2 were vaccinated with one dose of the Reo2 live vaccine (GC5) by intramuscular (IM) injection in the breast muscle. Groups 3, 4 and 5 were not vaccinated but were injected with sterile PBS using 0.5 ml/bird by IM injection. Three weeks later, at the age of 9 weeks (63 days of age), Groups 1, 2, 3, and 4 were challenged according to the protocol summarized in Table 2.

   Group 5 remained as the non-vaccinated, non-challenged control group and the birds in this group were injected with a sterile solution of PBS, using the same volume as the challenge groups.

   The challenge of both viruses was carried out by the foot-

Table 2: Summary of the vaccination and challenge protocol

Group number	Vaccination at 42 days of age	Challenge at 63 days of age Injection in foot pad using volume of 0.05 ml/bird	Number of Chicks/Group
1	REO2 Live vaccination	Homologous Challenge with Reovirus GC5	12
2	REO2 Live vaccination	Heterologous Challenge with Reovirus GC2	12
3	Not Vaccinated PBS injection	Challenge Reovirus GC5	12
4	Not Vaccinated PBS injection	Challenge Reovirus GC2	12
5	Not Vaccinated PBS injection	Non-vaccinated + non-challenged (PBS footpad injection)	12

Table 3. Lesion Scoring evaluation Parameters.

Veterinary scoring evaluation	Observation
0	Healthy leg
1	Minimal swelling of the footpad
2	Mild swelling of the footpad
3	Intense swelling of the foot pad but no swelling in the leg tendon
4	Intense swelling in the footpad and slight swelling in the tendons
5	Intense swelling in the footpad and the tendons of the leg,

pad injection in the right foot of the birds using a calibrated 1ml syringe and 21G needles.

5. **Serologic response after vaccination with the Reo2 live vaccine:**

   Blood samples were taken from the birds in the different groups at 42 days of age (before vaccination= Time 0) and 3 weeks later (63 days of age).

   The blood was collected from the wing vein and the serum was separated from the clot and kept at -20^{0}C until tested.

   All the samples from the vaccinated and non-vaccinated groups were tested by an In-house developed ELISA as follows:

   A. Recombinant Sigma C protein from REO GC5 was coated on 96 well NUNC Maxisorp plates (Thermo Fisher Scientific Inc.).

   B. Following blocking, the serum samples (from vaccinated and non-vaccinated chickens) were diluted 1:500 and distributed in the coated plate wells.

   D. Then, Donkey anti-Chicken IgY conjugated to HRP was added (Jackson ImmunoResearch Inc.).

   E. The color signal was detected by the addition of TMB substrate, and the reaction was stopped using an acid solution.

   F. S/P ratio [Sample-NC)/(PC-NC)] was calculated from absorbance values (O.D units) obtained at 450 nm in Agilent BioTek 800 TS Microplate Reader.

6. **Lesion Score Evaluation:**

   Following the challenge, the Lesion Score and protection level were evaluated by veterinary inspection (visual and palpation of the legs and footpads in both legs from each bird) at 3-, 7-, 10- and 14-days post challenge. (the visual and palpation *examination was carried out blinded by the veterinarian without any knowledge of the treatment in each group*).

   The Lesion Scoring was based on the evaluation of the swelling level and dissemination using a zero to five scoring as presented in Table 3.

RESULTS

1. **Immune response after vaccination with the Reo 2 live vaccine (GC5):**

   Blood samples taken from the different groups before the vaccination (Time=0) having an S/P (Standard/Positive score) value of less than 0.10 indicaed that the birds did not have any antibodies against the indicated GC5 Reovirus before the vaccination. A significant immune response was observed only in the birds vac-

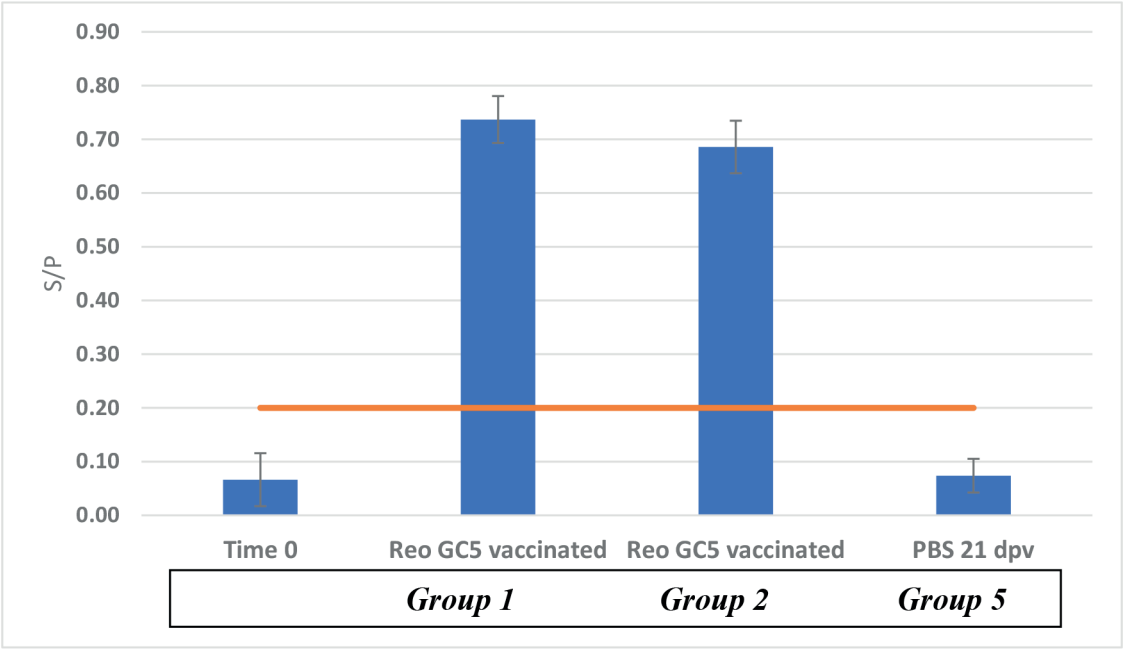


Figure 3: Antibody S/P levels in different groups (vaccinated and control groups)

cinated with the Reo 2 live vaccine and tested just before the challenge (three weeks post-vaccination). The vaccinated birds from groups 3 and 4 reached S/P values of about 0.7 compared to S/P less than 0.10 in the PBS-injected birds. Summary of the serologic results are presented in Figure 3.

2. **Lesion Score after challenge and evaluation of protection provided by the Reo 2 live vaccine against GC5 or GC2 Reoviruses:**

At four intervals following the challenge (3 days post challenge (dpc), 7dpc, 10dpc and 14dpc), both legs of the chickens were inspected, and scoring was made individually for each leg. In all the inspected chickens, the non-injected leg (left leg) received a score equal to 0 (data not shown) and compared to the injected leg. The scoring of non-vaccinated non-challenged group (Group 5) was minimal at each interval post-challenge (equal to 0). Both challenged |non-vaccinated control groups (Groups 3 and 4), reached their peak of Lesion Score at 10 dpc with significantly high scores of 1.33±0.25 in the GC5 challenged group, and 2.33±0.14 in the GC3 challenged group when compared to score=0 obtained in the PBS footpad injected control group (Group 5) and negligeable score obtained in the vaccinated groups (Group 1, Group2). The results of the Post challenge scoring are summarized in Figure 4.

DISCUSSION

Since the introduction of the controlled exposure vaccination in broiler breeder flocks in 2019, a significant reduction in the number of cases and the severity of the tendosynovitis in the broiler flocks was observed (11). Before the introduction in the field of the vaccination by controlled exposure using the non-attenuated Reovirus, all the breeding flocks were routinely vaccinated several times against Reovirus using monovalent or multivalent inactivated vaccines containing several genotypes of the Reoviruses isolated in Israel. Despite the extensive use of the inactivated vaccines, the reduction in the number of diagnosed cases of tendosynovitis and the severity of the cases was minimal (1, 10). Autogenous inactivated Reovirus vaccines usually contain the genotype clusters of Reoviruses isolated in certain area, but the main limitation of these vaccines is the relatively low titer of Reoviruses included in each dose of the inactivated vaccines, as field isolates of Reovirus do not reach viral titers that are sufficient to induce a significant immune response in the vaccinated birds. Reovirus live vaccines, as in the case of the (GC5) Reo2 live vaccine, require much lower vaccinating titers ($10^{3.5}$

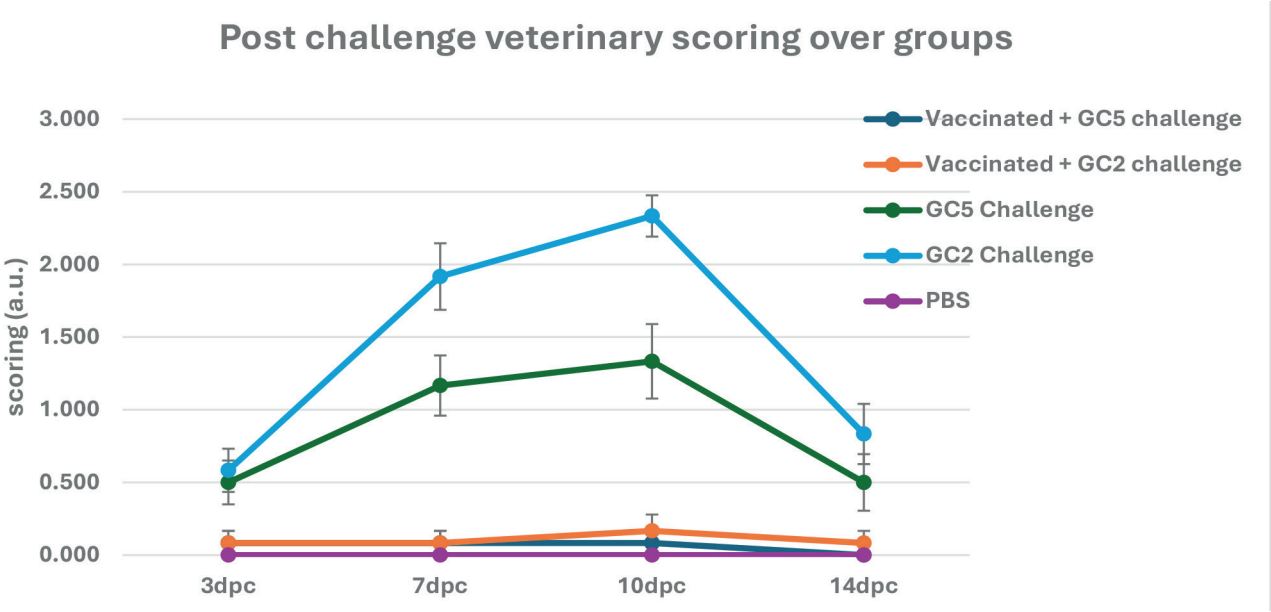


Figure 4. Lesion Score in the different groups.

EID_{50}) as the virus replicates and induces a strong cellular and antibody immune response.

Despite the genetic and antigenic differences between the Reoviruses GC5 and GC2, we did not observe any increase in the number of cases of Reovirus tendosynovitis caused by the GC2 as reported, after the use of autogenous inactivated Reovirus vaccines (3, 13), suggesting that vaccination with one genotype may cause the proliferation and spreading of a different genotype cluster. In Israel, since the introduction of the Reo2 live vaccine (GC5), the total number of cases and the severity of the clinical cases was reduced by more than 90% as reported by the Regional Poultry Diagnostic Laboratories in Israel, suggesting that the vaccination by controlled exposure with the GC5 (Reo2 live vaccine) may induce cross protection against the GC2 Reovirus.

The results obtained in this study, clearly demonstrate and corroborate the hypothesis that the vaccination of the breeding flocks with the Reo2 live vaccine (GC5) induced complete protection against the heterologous Reovirus GC2. The vaccination with the Reo2 live vaccine by IM injection, induced a clear and significant increase in the levels of antibodies that were able to prevent the replication and pathological damage after the homologous and the heterologous challenge with both Reoviruses.

The results of the lesion scoring showed that the GC2 Reovirus caused significant pathological changes after the footpad injection with significantly higher Lesion Score when compared to the non-vaccinated challenged with the GC5 Reovirus.

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