Research Article

Long Term Use Summary (2019-2021) of a Reovirus 2, Live Non-Attenuated Virus as Controlled Exposure Vaccination in Heavy Breeder Chickens in Israel

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ABSTRACT

Reovirus is the most important causal agent of arthritis/tenosynovitis in chickens, especially in heavy breeds. Early infection with Reovirus causes inflammation and scarring of the gastrocnemius and flexor tendons, leading to lameness, tendon rupture, and abnormal leg spreading. Arthritis/tenosynovitis leads to economic losses and critical welfare issues in the poultry industry in many countries. In Israel, the economic impact of the disease is higher due to the significant condemnation rates related to Jewish Kosher laws. Sigma C sequencing of isolated reoviruses in Israel demonstrated that the same group of reoviruses (Reovirus-Cluster 2 today defined as Reovirus GC-5) caused more than 95% of the cases reported in broilers and breeders since 2015. The use of commercial or autogenous Reovirus inactivated vaccines based on different strains of Reovirus, did not prevent the infection or the shedding of the virus to the progeny under field conditions. Extensive use of inactivated Reovirus vaccines only reduced the time of virus shedding to the progeny from 5-7 weeks before the introduction of the vaccines to 3-4 weeks of shedding. Controlled exposure of breeding flocks during the rearing period using a live non-attenuated Reovirus 2 live vaccine (GC-5) was tested in controlled laboratory and field trials during 2017-2018. Following successful controlled field trials, commercial implementation of vaccination using the non-attenuated Reovirus 2 live vaccine commenced in Israel in 2019. Since 2019, nearly 100% of the heavy breeders in Israel have been vaccinated during their rearing period. Close monitoring of all vaccinated flocks and their progeny was carried out between 2019 and 2021. No clinical complications or adverse reactions in any breeding flock following the use of the live vaccine were reported during the rearing period and production. A significant reduction in the number of clinical cases and Reovirus isolations were observed in breeding and broiler flocks following the introduction of the nonattenuated Reovirus 2 live vaccine. This report summarizes the information accumulated from the large-scale use of the controlled exposure method using the Reovirus 2 live vaccine.

Keywords: Poultry; Avian; Reovirus: Arthritis/Tenosynovitis; Vaccination; Poultry Welfare; Controlled Exposure.

INTRODUCTION

New emerging reoviruses appeared in many countries during the last decade, causing viral tenosynovitis and severe economic and welfare issues. (1, 2, 3, 4, 9, 10, 11, 12, 14). 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 (3). The σC protein of Reovirus is responsible for its attachment

to the cell receptors and induction of specific neutralizing antibodies (4, 5).

Control of reovirus tenosynovitis is based on the vaccination of breeding flocks during the rearing period. The lack of efficacy of commercial vaccines against the newly emerging strains of Reovirus led the industry to develop and use autogenous Reovirus inactivated vaccines based on homologous strains (1, 2, 3, 4, 6, 7, 8).

The epidemiological data provided by the regional diagnostic laboratories in Israel (Egg and Poultry Board Laboratories-EPB) clearly showed that since 2015 despite the extensive use of commercial and autogenous inactivated Reovirus vaccines, most cases of tenosynovitis in broilers were related to egg transmission after infection of the breeding flocks. Epidemiological studies carried out in Israel at the Kimron Veterinary Institute demonstrated that most of the cases of arthritis/tenosynovitis in broilers in Israel were caused by reoviruses related to cluster 2 or as defined today, GC- 5 (16).

The accumulated economic damage caused by the Reovirus tenosynovitis in Israel reached almost one billion NIS in the last decade.

To mitigate the negative impact of reovirus arthritis/tenosynovitis in broilers in Israel, a novel and unconventional approach was developed and tested under controlled laboratory and field conditions and the preliminary results by Perelman *et al*. were published in 2019 (17).

The Israeli Veterinary Services approved controlled exposure of the broiler breeder pullets by intramuscular injection (IM) vaccination at around ten weeks of age with the non-attenuated Reovirus 2 Live Vaccine based on the Isolate #7585.

Since 2019 close to 100% of the broiler breeder flocks were vaccinated during the rearing period (8-12 weeks of age) with the new non-attenuated Reovirus 2 live vaccine as a controlled exposure vaccination. Monitoring of broiler breeders during rearing and laying periods and their broiler progeny performances and health status was carried out to detect any adverse reactions resulting from the Reovirus 2 live vaccine implementation. From 2019 to the end of 2021, approximately 8 million breeders (290 flocks) were vaccinated with the Reovirus 2 live vaccine, and these breeding flocks produced more than 600 million broiler chicks. The clinical and epidemiological results in broilers and broiler breeders are presented.

MATERIALS AND METHODS

1. Lines of breeding birds used in this study: Ross 309 and Cobb 500.
2. Reovirus 2 live vaccine was produced commercially at Abic Biological laboratories Israel (Phibro-Abic Israel). The vaccine was developed from a field isolate, of Reovirus cluster 2 (#7585) as classified by Goldenberg *et al*. 2010, according to a new updated adopted classification (16), the Reovirus Cluster 2 is considered as Genotype Cluster 5 (GC-5). The virus was adapted to grow in SPF chicken embryos. The vaccine titer was determined as $10^{3.5}$ EID 50/dose/bird. The Isolate and the Master seed of the vaccine were tested for extraneous agents before producing commercial batches.
3. The commercial vaccine virus was preserved in vials as a frozen vaccine at -80°C and provided to the farms on request no more than seven days before using the vaccine. Details of farms and flocks were recorded on a designated form. At the farms, the vaccine vials were kept at -20°C and the vaccine thawed just before use. Each 1000 doses vial was diluted in 500 ml sterile saline solution to provide one dose in 0.5 ml of the diluted vaccine per bird. The vaccine was applied by intramuscular injection to the breast.
4. Reovirus-2 Live vaccination schedules: Since the vaccine contains a non-attenuated live Reovirus, developing a vaccination protocol based on a controlled exposure strategy ensuring the safety of the vaccinated birds and their progeny was required. The vaccination strategy was based on the following parameters:
   a. The vaccine should be used only in approved pullet farms (rearing from day one to 23 weeks of age) with a high level of biosecurity.
   b. The vaccine should be applied to the pullets (females and males) at the age of 8-12 weeks when the skeleton had completed its development to reduce potential damage to the tendons due to fast-growing during the first weeks of age.
   c. The vaccine must be applied by intramuscular injection to the breast muscle of all the birds on the farm to provide a uniform vaccination (controlled exposure). Intramuscular vaccination potentially limits the invasiveness and shedding of the virus thus preventing a rolling infection effect and po-

tential pathogenicity and the spreading of the virus. Previously published data by Perelman *et al.*, 2019 (17), demonstrated that the injected Reovirus was shed in the feces for a short period for about one week.

d. Monitoring the vaccinated pullet farms included recording any clinical sign that could indicate an adverse effect of the vaccine to the vaccinated birds (any leg or locomotion problem, feed consumption, growth rates, or increased mortality). The Reovirus-2 live vaccine application was included in all the breeding farms as a complementary part of the vaccination programs which included the use of polyvalent and monovalent autogenous Reovirus vaccines applied 2-3 times according to vaccination programs applied for each organization.
e. After transferring the replacement pullets to the designated breeding farms, the broiler breeders and their progeny were clinically monitored. Veterinarians, farms, and hatchery managers were instructed to report any adverse effect in the breeding birds, production of fertile eggs and any problem related to tenosynovitis or reduced growth rates in the broiler progeny.
f. In any reported case of tenosynovitis in the progeny (broilers) of vaccinated flocks, an epidemiological investigation was carried out to determine the source of infection of the reported case.
g. Infection of a breeding flock and vertical shedding was considered as positive when reovirus tenosynovitis occurred in at least two consecutive hatches of chicks or when the clinical signs of tenosynovitis appeared in the progeny from week one and up to three weeks of age.
h. In suspected Reovirus infection cases, samples of organs or affected birds were sent to the regional laboratories for virus isolation and to Kimron Veterinary Institute for PCR detection, virus isolation and classification.

RESULTS

Large scale vaccination of the breeding flocks with the Reovirus-2 live vaccine started in Israel in 2019. Between April 2019 and December 2021, 296 flocks including 8 million breeders (100% of the breeding flocks in Israel) were vaccinated with the Reovirus-2 live vaccine during the rearing period as described before (see Table 1).

These breeding flocks produced more than 650 million broilers; the Reovirus-2 live vaccine was applied to the breeder rearing flocks between 8-12 weeks of age depending on the vaccination programs of the farms.

Safety and adverse reactions after vaccination of the Reovirus-2 live vaccine:

a. **In rearing flocks:** No reports of tenosynovitis or leg disorders, reduced growth rates, change in feed consumption or other signs suggesting adverse reactions were reported in any of the rearing flocks after vaccination with the Reovirus-2 live vaccine.
b. **In Breeding flocks:** No reports of any adverse effects such as increased mortality (culling) in females or males, reduced egg production, fertility chick quality or any other adverse effects were reported in any of the vaccinated flocks.
c. **Hatcheries and Progeny (broilers):** No reports of adverse effects such as embryo mortality, reduced fertility, hatchability, or percentage of grade A chicks were reported from any of the hatcheries incubating eggs originating from vaccinated breeding flocks.

Efficacy: Inactivated autogenous Reovirus vaccines were introduced in 2016 as the first option to try to reduce the welfare and economic damage caused by the tenosynovitis and condemnations caused by the vertical shedding of the Reovirus from the breeding flocks to the progeny (broiler flocks). Different autogenous polyvalent (Reovirus Clusters 1, 2, 3, 4) inactivated vaccines were introduced in the vaccination programs of the breeding flocks starting 2016. These inactivated vaccines were injected two or three times during the rearing period of the replacement pullets according to different vaccination programs. From 2015 to 2019 the number

Table 1: Number of doses supplied of Reovirus 2 live vaccine (Isolate# 7585 Reovirus Live) and number of farms using the vaccine each year (Phibro-Abic marketing records).

Year	No of Farms	Doses of Vaccine
2018	2 (Field Trials)	60,000
2019	73	2,111,000
2020	106	3,225,000
2021	112	2,928,000
Total	293	8,264,000

of tenosynovitis cases in broilers remained about the same with most of the cases due to vertical transmission from the infected breeding flocks to the progeny. Reovirus Cluster 2 inactivated vaccine or polyvalent autogenous inactivated vaccines, helped apparently to reduce only the length of time of the vertical shedding but did not prevent the infection of the breeding flocks or the vertical shedding of the virus. The summary of the reported cases and isolation of Reovirus in Israel is represented in Figure 1.

Evaluation of the efficacy of the vaccination of breeding flocks with the Reovirus-2 live vaccine was based on the epidemiological data collected during the period between April 2019 to the end of 2021. During this period 100% of the replacement pullets in Israel were vaccinated with the Reovirus-2 live vaccine. The Reovirus-2 live vaccine was added to the vaccination program of every breeder and hatchery organization and included the Ross 308 and Cobb 500 poultry lines.

The vaccine was applied by intramuscular injection in the breast muscle in all males and females on the farms.

A gradual but consistent drop in the cases of Reovirus tenosynovitis was observed during the introduction period between April 2019 to April 2020. During this period about 86 breeding flocks were in production, 40 older flocks (46%) were non-vaccinated, and 46 flocks (54%) were vaccinated with the Reovirus-2 live vaccine at the rearing farms. During this period, 5 breeding flocks were infected with Reovirus producing a total of 53 cases of Reovirus tenosynovitis in broiler flocks (Table 2). All the cases reported occurred only in non-vaccinated breeding flocks while none of the flocks vaccinated with the Reovirus-2 live vaccine were found positive for Reovirus or shedding to their progeny.

Since April 2019 to December 2021 no cases of Reovirus tenosynovitis isolation or detection have been reported in any of the breeding flocks vaccinated with the Reovirus-2 live vaccine in Israel. During this period of time, a significant reduction in the number of cases of tenosynovitis was reported in broiler flocks (from 118 cases in 2018 to 13 cases by the end of 2021). According to the epidemiological data collected by the Regional Poultry Laboratories (Figure 2), the cases of tenosynovitis in broiler flocks fluctuated each year between 2015 to 2019. The number of cases of reovirus tenosynovitis in broilers in 2020 and 2021 was considerably lower when compared to previous years and the economic impact was reduced from more than 100 affected broiler flocks with very high condemnation rates/year to 13 cases with very low condemnation rates.

DISCUSSION

A preliminary report on the safety and efficacy of the Reovirus-2 live vaccine for breeding flocks under controlled conditions was published in 2019 (17).

In this report, we included the updated epidemiological data obtained during the period between April 2019 and December 2021 after the introduction of the Reovirus-2 live vaccine on a large scale in Israel including more than 8 million breeders in 290 breeding flocks and more than 650 million broilers. According to all accumulated data, it can be

Table 2: Number of Reovirus isolations and cases as reported by the Regional Laboratories - Egg and Poultry Board (EPB) from April 2018 – December 2021.

	Apr 2018-Apr 2019	**Apr 2019-Apr 2020**		**Apr 2020- Dec 2021**
	100% of breeding flocks vaccinated only with **inactivated Reovirus vaccines**	Vaccinated only with **inactivated Reovirus vaccines**	Vaccinated with **Reovirus-2 live +** Reovirus inactivated vaccines	100% breeding flocks vaccinated with Reovirus-2 live and Reovirus Inactivated vaccines
		Number of breeding flocks 40	Number of breeding flocks 46	Approximately 130 active breeding flocks
Number of cases (Isolates) reported in broilers	118	53	None	13
Number of cases (Isolates) in breeding flocks	6	5	None	None
Number of cases in broilers related to breeding flocks	118	53	None	3 cases from the same breeding flock, related to technical problems of vaccine application in the breeding flock

concluded that the use of the Reovirus-2 live vaccine during the rearing period in the replacement pullets proved to be safe for the pullets, the breeders and the progeny.

The vaccination of the pullets at the recommended age of 8-12 weeks did not cause any adverse reactions in the vaccinated birds. The use of the live Reovirs-2 vaccine resulted in a significant reduction in the cases of tenosynovitis in broiler chicks compared to the period before the introduction of

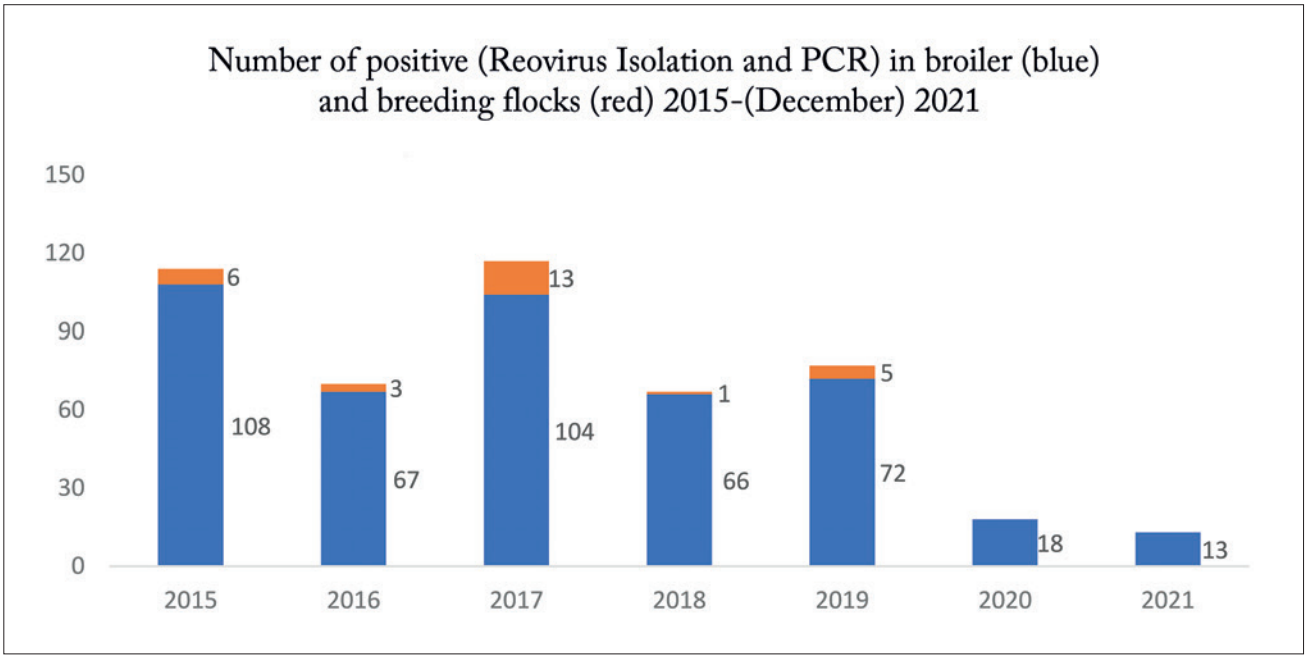

Figure 1: Data representing Reovirus Isolation in breeding and broiler flocks in Israel from 2015 to the end of 2021 (Data provided by Egg and Poultry Board Regional Laboratories).

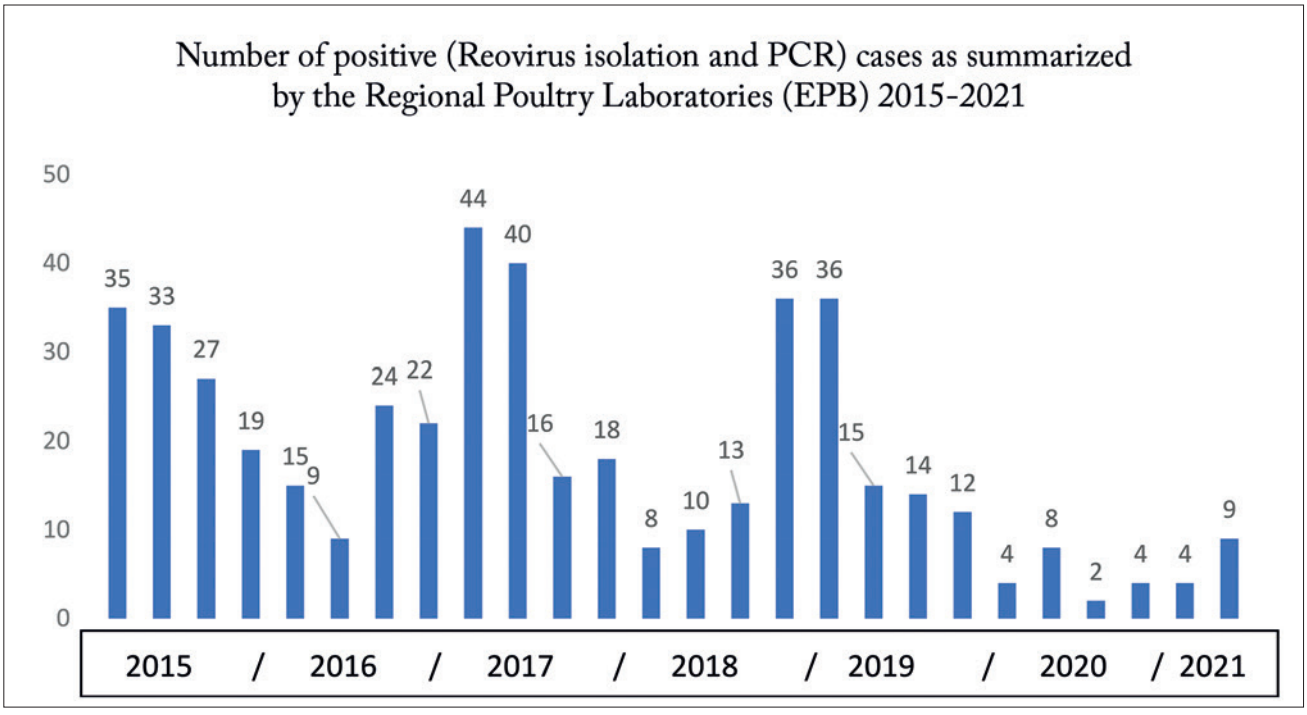

Figure 2: Number of isolation and detection of Reovirus in broiler flocks by year (quarterly) from 2015 to December 2021.

the vaccine. Before the introduction of the Reovirus 2 live vaccine, the fluctuations in the number of clinical reovirus tenosynovitis cases were related to reovirus infections in new breeding flocks and the vertical shedding of the virus to the progeny for several weeks, with only a few cases of horizontal infection.

Since the introduction of the Reovirus-2 live vaccine these fluctuations were much lower as no cases of reovirus were reported or diagnosed in any of the vaccinated breeding flocks and the vertical shedding of the broiler progeny seemed to be prevented by the vaccine. It should be pointed out that the vaccination program used, only provided protection to the progeny by maternal antibodies for about 14 days, after which local infection with Reovirus in contaminated premises may occur.

One of the biggest problems we still face is the lack of specific and accurate serologic tests to determine the immune response of the birds after vaccination with autogenous inactivated Reovirus-2 or Reovirus-2 live vaccine. Commercial ELISA tests are based on Reoviruses of cluster I such as the 1133 and detection and titer measurement of specific antibodies produced by the Reovirus 2 live vaccine (GC-5) is very limited in these commercial kits.

It seems that the specific antibodies produced after vaccination with the Reovirus-2 live vaccine can provide protection to the breeding flocks for the production period and to the progeny up to 2 weeks of age, depending on the levels of the maternal antibodies. All the reported cases in 2020 and 2021 were found in broilers only at the slaughterhouse, without any clinical signs during the rearing or growing period. Further studies are planned to prove the efficacy and duration of immunity of the maternal antibodies in broiler chicks originating from vaccinated breeding flocks. A specific ELISA test for the reovirus Cluster 2 (GC 5) is still in development and will be used to test the immune response of the birds after vaccination with Reovirus 2 live and inactivated vaccines and their combinations. It will also help to develop better vaccination programs for the control of reovirus.

This report supports the approach of using live embryo or tissue culture adapted non-attenuated emerging Reoviruses for vaccination of breeding birds during the rearing period to reduce the negative economic and welfare impacts of these emerging viruses.

The application of the Reovirus-2 live vaccine by intramuscular injection (IM) in replacement pullets at the age between 8-12 weeks on the rearing farms, proved to be safe for the birds at the farm and the surroundings, as the uniform vaccination by IM injection induced a good protection and prevented the rolling infection of the virus.

It can be concluded based on the data accumulated after the large-scale use of the Reovirus-2 live vaccine on many millions of breeding birds and their progeny that the vaccine is safe and efficient as an aid in the reduction of the infection and shedding of the reovirus in vaccinated breeders and their progeny.

The use of the Reovirus-2 live vaccine and the vaccination approach based on "controlled exposure" provided significant and momentous positive welfare and economic influences to the poultry industry in Israel.

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