Use of Controlled Exposure as a Novel Method for Reovirus Arthritis/Tenosynovitis Prevention. A Preliminary Report.

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ABSTRACT

Viral Avian arthritis/tenosynovitis has become in the last decade a serious economic threat to the poultry industry. Reovirus is the most important causal agent of arthritis/tenosynovitis in chickens. The early infection with reovirus causes inflammation and scarring of the gastrocnemius and flexor tendons, causing lameness, tendon rupture and deviation of the legs. Arthritis/tenosynovitis in chickens is not only a serious economic burden but also an important welfare problem to the poultry industry in many countries. Available commercial live and inactivated reovirus vaccines contain reovirus strains that belong to the same serotype (S-1133 or 1733). These strains, are antigenically different from the new emerging reoviruses and do not provide any protection against infection of the breeding flocks and transmission of the virus to the progeny. The new emerging reoviruses are more pathogenic and vertically transmitted, causing severe arthritis/tenosynovitis in broilers and breeders as young as 14 days of age. Sigma C sequencing of isolated reoviruses in Israel, demonstrated that the same group of reoviruses has been found to cause more than 90% of the cases. In order to reduce the economic and welfare impact of the disease in broiler and breeder flocks, a novel approach based on controlled exposure of the breeding flocks during rearing with a wild type live reovirus was tested. The controlled exposure of the flocks did not cause any damage to the health of the pullets, breeders or the production of eggs and chicks. The progeny of the vaccinated flocks did not show any signs of reovirus infection throughout their full production period.

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

INTRODUCTION

Viral Avian arthritis/tenosynovitis caused by avian reovirus (ARV) belongs to the orthoreovirus genus of the Reoviridae family. During the last decade, new emerging reoviruses has appeared in many countries causing economic damage to the poultry industry and severe welfare issues. Meat type chickens including broilers and breeders are the most affected (1, 2, 3, 4, 9, 10, 11, 12, 14), but in some countries such as the USA, reoviruses also affects turkey poults causing serious economic losses (1, 2).

Genetic diversity among ARV strains occur 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 of the virus to the cell receptors and for induction of specific neutralizing antibodies (4, 5).

Reoviruses in general may infect chickens at any age by horizontal or vertical transmission: broiler breeders infected during the laying period transmit the virus to their progeny via eggs, mostly without any reduction in egg production

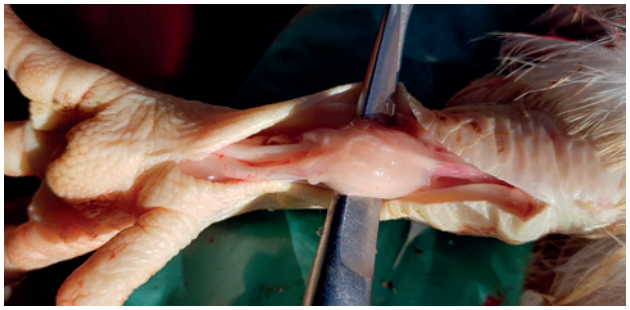


Figure 1: Swollen tendons due to early infection with reovirus cluster II in a 3-week-old broiler chicken

or clinical signs. The infected chicks may then demonstrate varying degrees of clinical and pathological arthritis/tenosynovitis signs (mild – severe) as early as 12 to 14 days of age with morbidity rates as high as 60%. The main clinical signs in affected flocks of broilers are lameness, reluctance to move, inflammation and swelling of the gastrocnemius and digital flexor tendons (Figure 1) and deviations of the tarsus (Figure 2).

Severity of reovirus arthritis/tenosynovitis will depend on pathogenicity of the reovirus, age of infection and immune status of the flock. Chickens infected vertically via the egg, or at one day of age by cross contamination at the hatchery, may show early and severe clinical signs, while birds infected after 7 to 15 days of age may develop mild arthritis/tenosynovitis with very few clinical signs or subclinical disease (10) but high condemnation rates in the slaughterhouse.

Rupture of the tendon occurs due to inflammation, scarring and the loss of elasticity of the tendon (Figure 3). In broilers, there is a strong correlation between the rapid growth and increase of body weight to condemnation rates in the slaughterhouse due to ruptured tendons.

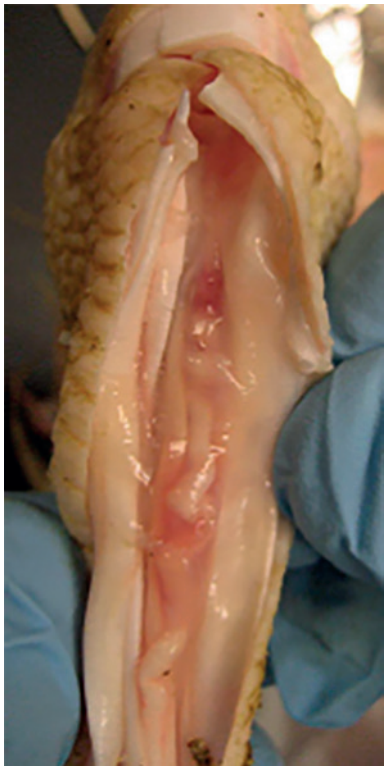


Figure 3. Rupture of tendons after infection with Reovirus cluster II

Jewish Kosher laws defines birds with inflamed, damaged or torn gastrocnemius and digital flexor tendons as non-Kosher and since most of the slaughterhouses in Israel follow Kosher regulations, value losses can rise to 60% for a whole flock.

Epidemiological data provided by the regional diagnostic laboratories of the Egg and Poultry Board in Israel (EPB), clearly showed a drastic increase in clinical cases of arthritis/tenosynovitis and isolation of reovirus from broilers at very young age since 2015 (Figure 4 and Figure 5). It is important

Figure 2: Deviations of the legs in 14 days old broiler chicken infected with (vertical) egg transmitted Reovirus cluster II.

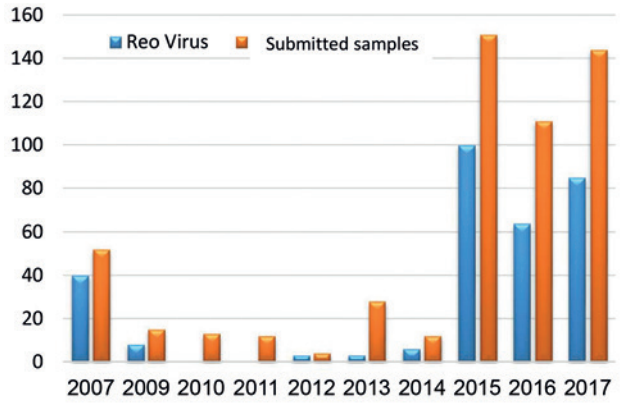


Figure 4: Total number of submitted samples and Reovirus isolated from clinical and condemnation cases in Israel from 2007 to 2017. EPB laboratories

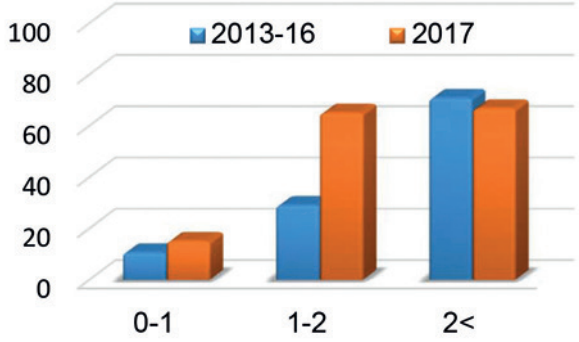


Figure 5: Isolation rates of Reovirus from clinical cases according to age of broiler chickens from 2013 to 2017. EPB Laboratories

to emphasize that from 2007 to 2014, high condemnation rates due to damaged tendons (non-Kosher) where observed mainly at the slaughterhouse with very few samples being sent to the diagnostic laboratories for identification of reovirus (personal communication).

In Israel as well as in other countries, control of reovirus Avian arthritis/tenosynovitis, is based on the vaccination of the breeding flocks during the rearing period. The only current commercial live and inactivated vaccines contain the 1133 and the 1733 strains of reovirus that belong to cluster I. These vaccines have been used worldwide during the last decades. However, the lack of efficacy of these vaccines against the newly emerging strains of reovirus has forced the industry to try to develop and use more homologous strains including autogenous inactivated reovirus vaccines (1, 2, 3, 4, 6, 7, 8).

Genotype classification of reoviruses based on the sequencing of the Sigma C (σC) of new isolated reoviruses, clearly indicates that the new isolated viruses belong to different clusters with significant antigenic differences (7, 8) when compared to the current vaccine strains.

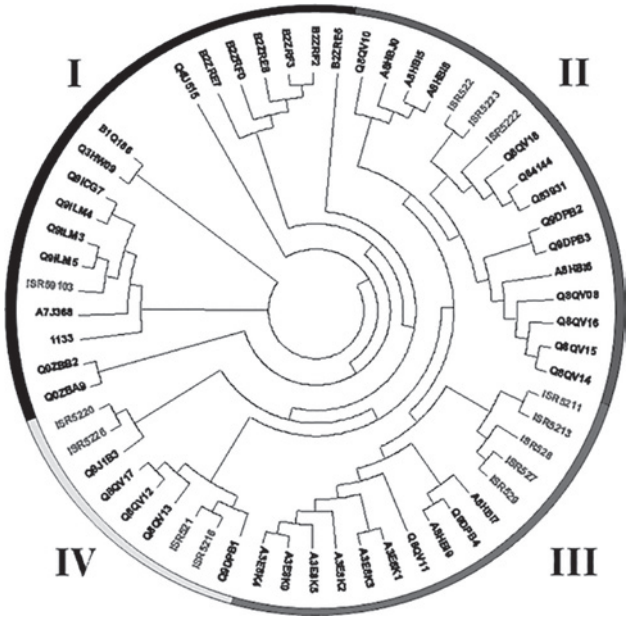
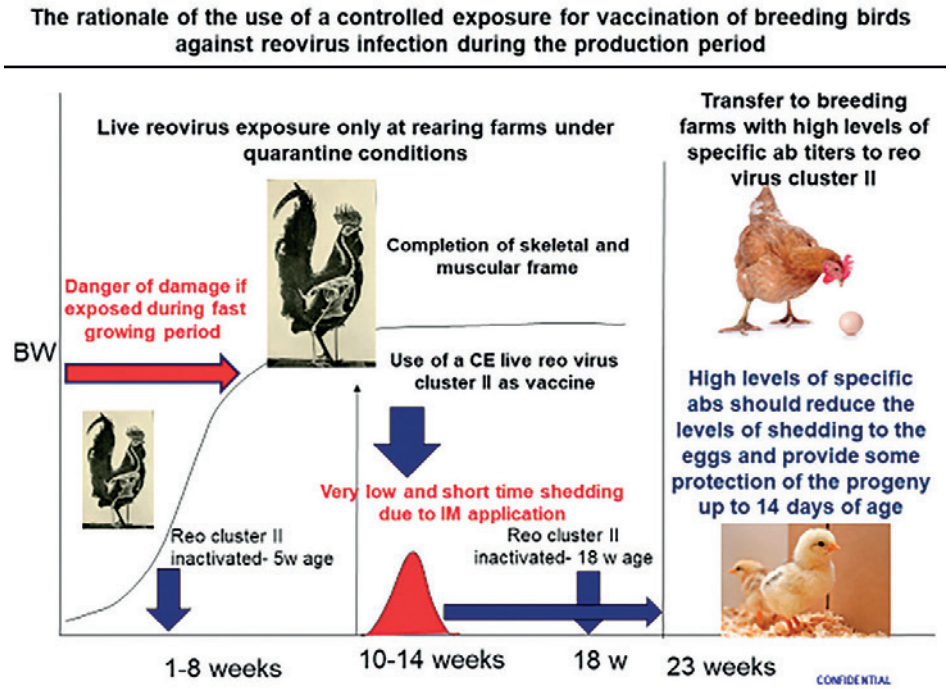


Figure 6: Phylogenetic tree of ARV strains according to variability in their Sigma C protein sequences (8)

Based on sigma C sequence analysis, ARV isolates were clustered into 4 groups (8). Epidemiological studies carried out in Israel by Dr. Farnoushi from the Kimron Veterinary Institute during the last few years demonstrated that most of the cases of arthritis/tenosynovitis in broilers in Israel were caused by reoviruses belonging to cluster II while the current commercial vaccines contain reoviruses 1133 and 1733 belonging to cluster I (personal communication). (Fig. 6)

Autogenous inactivated reovirus vaccines containing a reovirus from cluster II, has been found unable to induce adequate antibody titers and were only able to reduce the time of virus egg transmission. Unfortunately, despite the intensive use of these autogenous inactivated vaccines (2-4 applications); the economic impact due to vertical and cross contamination causing non-Kosher condemnation rates remained very high (Personal communication).

In order to mitigate the negative impact of reovirus arthritis/tenosynovitis in broilers, a novel and unconventional approach was developed and tested. The new concept was based on the controlled exposure of the heavy breeder pullets



Chart

with the live chicken embryo (CE) adapted reovirus cluster II (#7585) at around ten weeks of age, The live CE adapted cluster II reovirus was used for breeding flocks vaccination in order to induce a better immune response and protection to the progeny.

The vaccination concept, based on a controlled exposure to a live CE adapted reovirus is based on the following rationale:

1. Reovirus infection causes severe clinical and pathological arthritis/tenosynovitis only when the chicks are infected at a very young age (10, 14). Reovirus infection of chickens when the musculoskeletal frame is almost fully developed (8-10 weeks of age) causes a subclinical infection with little or no damage to the flock as reported (10, 14).
2. The use of a low dose of a specific reovirus (cluster II), adapted to CE or chicken embryo fibroblasts (CEF) as a live vaccine, and administered by intramuscular injection (IM), ensuring that every chicken receives the same dose of live reovirus vaccine. The viremia following vaccination may induce a much better immune response and higher antibody titers.
3. The intramuscular injection was preferred in order to reduce the chance of excessive virus shedding in the feces and "rolling" of the virus within the flock, which could be the case if the vaccine was administered by the oral route with high and uncontrolled replication and shedding of the virus from the intestinal tract.
4. Under commercial conditions, the preliminary use of an inactivated reovirus cluster II vaccine at 5 weeks of age, was intended to induce a basic immune response before the application of the live cluster II (#7585) reovirus, reducing the possibility of any potential damage caused by the live virus injection. The second vaccination with the inactivated reovirus cluster II at 18-20 weeks was intended to be used as a booster in order to further increase the specific antibody titers to reovirus cluster II during the egg production period as summarized in the presented chart.

MATERIALS AND METHODS

The protocol for this study was approved by the Isaeli national council for animal experimentation. The permit numbers given by the council were IL-16-04-96 and IL-18-5-141.

The study was carried out in two stages.

Stage 1. ***Safety and virus shedding after vaccination of breeding birds by controlled exposure under laboratory conditions.***

The first stage goal was to ensure the safety of the procedure (controlled exposure by intramuscular (IM) injection) to growing unvaccinated or naturally exposed breeders at 7 weeks of age. The age of administration was selected as the time when the musculoskeletal frame is almost fully developed. Intramuscular injection was selected in order to deliver an accurate dose of the reovirus to each bird; to increase safety by avoiding "rolling" of the virus within the vaccinated group and thirdly by introducing it to the immune system through an unnatural route of infection in order to reduce the shedding levels of the live virus.

Intensive clinical monitoring of the vaccinated birds was carried out from day of vaccination to production at 23 weeks of age. Real time PCR was used to evaluate viremia and the shedding of reovirus after vaccination.

Vaccine virus.

Reovirus virulent Isolate No. 7585-cluster II (Abic-Biological Laboratories-PHIBRO, Israel) was adapted to Chicken Embryo fibroblasts (CEF) tissue culture and SPF chicken embryos (CE) and used as a vaccine for reovirus cluster II. The Master seed virus used, was extensively tested for foreign infectious agents including: Chicken anemia virus, Avian Leukosis virus, Reticuloendotheliosis virus, Marek disease virus, Avian Encephalitis virus, *Mycoplasma* spp., *Chlamydia* spp. and *Salmonella* spp.

The content of the seed virus was tested using specific real-time RT-PCR to cluster II and sigma C segment sequenced in order to corroborate that the only reovirus present in the vaccine was the Isolate No. 7585 of cluster II.

The virus in the "vaccines" (CEF-tissue culture or CE replication) was titrated to obtain $10^{3.5}$ EID/dose/bird and frozen at – 70^{0}C in vials of 1000 doses. At time of vaccination, every 1000 dose frozen vial was diluted in 500 ml sterile PBS to obtain one dose/bird in 0.5 ml. The vaccine was applied by intramuscular (IM) injection in the breast muscle.

Sensitive one step SYBR Green based Real Time RT-PCR (Cat. BIO-73005, Bioline London, United Kingdom) with melting curve analysis was developed to detect ARV cluster II isolates. We used fifty different ARV isolates between the years 2015 and 2018 stored at the department of avian diseases in Kimron Veterinary Institute. Each sample was collected from broiler chickens exhibiting arthritis, tenosynovitis and other signs associated with ARV infection. Tendons and joints from affected broiler chickens were minced with a scalpel and homogenized in PBS to a concentration of 1:5 weight/volume. These samples were centrifuged at 2500 rpm for 10 min at 4˚C. Finally, the supernatant was collected and filtered through a 0.22 ηm syringe filter, and inoculated into the yolk sac of SPF eggs. After incubation of inoculated eggs for 5-7 days, the allantoic fluid was collected for RNA extraction. Viral RNA was extracted using RNA easy Mini Kit (Cat. 74106, QIAGEN, Hilden, Germany), following the manufacturer's instructions. The extracted RNA was used as a template for SYBR Green Real Time RT-PCR. One pair of primers was designed from the conserved region of Israeli cluster II sigma C segment. These primers produced an 89 bp fragment, and used for SYBR Green Real Time RT-PCR, sequences of primers designed for the study are presented in table 1.

Table 1: Primers designed for this study from the conserved region of the Israel Cluster II sigma C segment

Name	Gene Target	Sequence
ARV-C2F	Sigma C	5′-TCATCGCAGGGACTTACAATC-3′
ARV-C2R	Sigma C	5′-GGGATCTGCAAACGAAAGAG-3′

Each PCR reaction used 4 μL of RNA, 10 μL of 2x SensiFAST™ SYBR® Hi-ROX One-Step Mix (Cat. BIO-73005, Bioline London, United Kingdom), 0.8 μL of each 10 μM primer, 0.2 μL Reverse transcriptase, 0.4 μL Ribosafe RNase inhibitor and 3.8 μL of H_2O to reach the total reaction volume of 20 μL. The number of cycles and the cycling times were configured using the 3-step cycling method of SensiFAST™ SYBR ® Hi-ROX One-Step Kit. Manufacturer's instruction as follows: One cycle of 45˚C for 10 min for reverse transcription, one cycle of 95˚C for 2 min for polymerase activation, and then 40 cycles at 95˚C for 5 sec for template denaturation, 60˚C for 10 sec for annealing and 72˚C for 5 sec for extension.

To plot the dissociation curve (melting curve), the following conditions were used: 95˚C for 15 sec, 60˚C for one minute and then heating slowly at 0.3˚C/sec until 95˚C for 15 sec. To confirm the presence and purity of amplicons, PCR products were resolved on a 3% agarose gel, stained

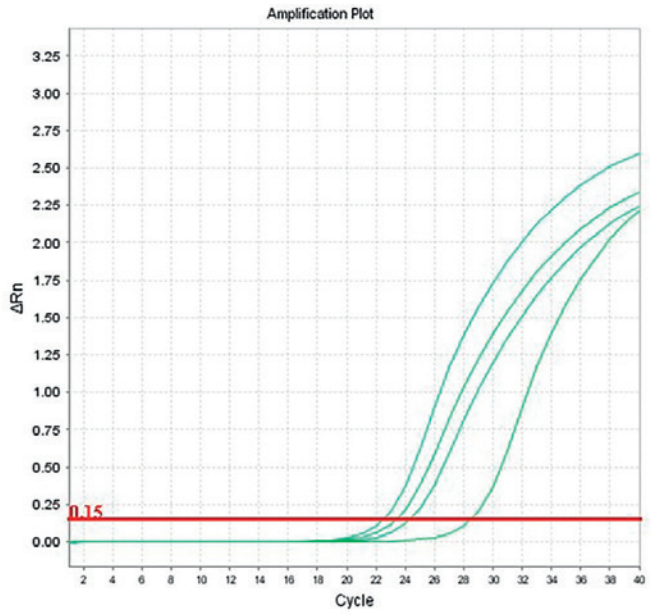


Figure 7: Cycle time amplification of several reoviruses cluster II

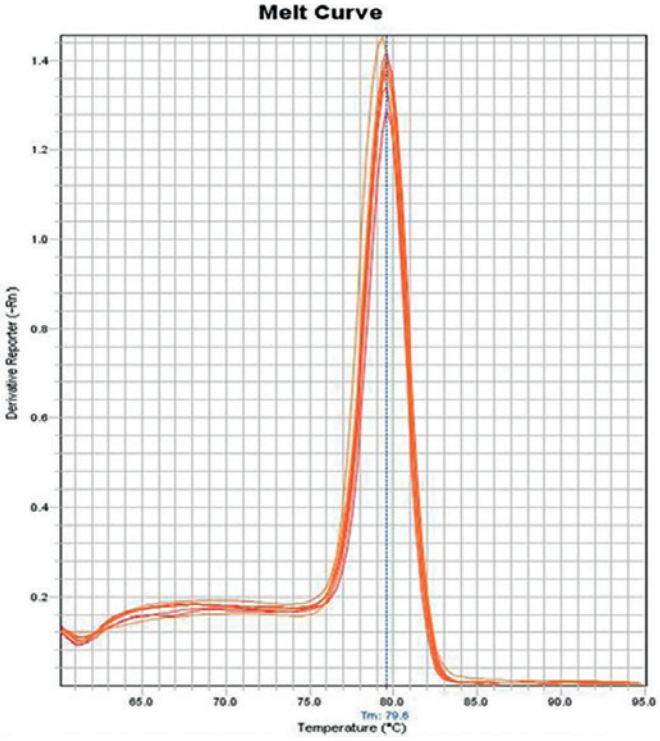


Figure 8: Single distinct peak in the melt curve plot

with ethidium bromide and photographed. To determine the specificity of Real Time RT-PCR, this reaction was run using samples from other viruses such as infectious bronchitis virus (IBV), infectious bursal disease virus (IBD), Newcastle disease virus (NDV) and ten healthy organ samples.

All 50 samples were amplified by real-time RT-PCR. The samples did not show alterations in the linear or logarithmic amplification curves, and no dimers were amplified in any reaction (Figures 7 and 8).

Birds used and safety monitoring protocol under laboratory-controlled conditions.

In order to evaluate the safety of the controlled exposure – to Reovirus cluster II for breeding birds, two hundred Ross breeding pullets, unvaccinated and unexposed to any known reoviruses (pretested by serology and real-time RT-PCR), were acquired from a commercial pullet farm (Yavne breeding and hatchery company, Israel). The two hundred birds were composed of about 85% females and 15% males.

After being tested by real-time RT-PCR for reovirus and by ELISA to determine that the birds had not been exposed to any known reovirus, the birds were transferred from Yavne rearing farm to PHIBRO experimental farm at Moshav Herut, Israel. The birds were separated in two groups of 100 birds each, keeping the same ratio of males to females in both groups.

At 7 weeks of age all the chickens in both groups were retested by real-time RT-PCR, wing numbered and bled for serology. All the birds in both groups were vaccinated (controlled exposure) by IM injection in the breast muscle with the live Reovirus (Isolate No. 7585). Group 1 received the CEF-tissue culture adapted virus. Group 2 received the CE-Embryo adapted virus of the same isolate.

All the birds in both groups were clinically monitored from the first week after vaccination up to 24 weeks of age, in order to detect any problems related to the vaccination. The clinical monitoring included physical inspection of both legs in order to detect any inflammatory processes in the joints, the tendons or the footpads. Monitoring of body weight, feed consumption and behavior. Monitoring and clinical inspection were carried out, from day of vaccination up to 24 weeks of age.

The birds in both groups were maintained under management and feeding conditions similar to those kept in commercial rearing farms, according to the Ross manual for the 308 Ross breeders.

Bloods for serological immune response to reovirus

vaccination were collected from 25 birds from each group at 7 (pre-vaccination) and 10 weeks of age (3 weeks post-vaccination). The serum was separated and tested using the IDEXX Reo ELISA test (IDEXX Laboratories, Westbrook, Maine, USA) for detection of anti-reovirus antibodies.

Viremia and virus shedding, in blood and feces were determined by real-time RT-PCR test, carried out from the day of vaccination. Blood and cloacal swabs from 25 bird from each group, were taken every week for the first 6 weeks after exposure and up to 23 weeks of age. The samples were sent the same day of sampling, to the Department of Avian Diseases at the Kimron Veterinary Institute, Israel.

By the age of 20 weeks, at start of egg production, all the females in both groups were challenged with the same reovirus (isolate No. 7585) at a dose of $2x10^{4.5}$ EID_{50}/bird by eye drop inoculation. Samples of blood, cloacal swabs and eggs were taken weekly from 20 weeks to 23 weeks of age in order to determine the shedding of the virus in the feces and the eggs. All samples, were sent to the Kimron Veterinary Institute, and tested by real-time RT-PCR for Reovirus cluster II.

Stage 2. *Safety and shedding of the Reovirus cluster II (#7585) after vaccination by controlled exposure, under commercial controlled conditions in rearing pullet farms.*

A special permit from the Veterinary Authorities in Israel was obtained to test the safety and risk of shedding of the Reovirus cluster II live virulent virus (#7585-cluster II), using the controlled exposure as a vaccination approach. All birds males and females were vaccinated with the chicken embryo adapted (CE) reovirus II at a final dose of $10^{3.5}$ EID_{50}/bird

Two rearing pullet farms were approved as quarantine farms, to test the novel approach under commercial but controlled and monitored conditions. The chosen rearing farms were located in relatively isolated areas with no broiler or breeding flocks in the vicinity. The first rearing pullet farm received 38,700 one-day-old Ross-308 chicks. The chicks were allocated in four houses with controlled ventilation under normal management conditions as recommended in Ross 308 rearing manual. The second rearing farm received 26,000 one-day-old Ross 308 breeding chicks and rearing conditions as recommended in the Ross 308 manual. Routine vaccination program at the farms included live and inactivated vaccines against: Marek, Newcastle, Infectious Bronchitis, Laryngotracheitis, Avian Encephalomyelitis, Avian Pneumovirus, Chicken Anemia Virus and Salmonella.

The reovirus vaccination program in the rearing farms included: Reo cluster II inactivated vaccine (Abic-PHIBRO, Israel) at 5 weeks of age, CE-Reovirus Cluster II-live vaccine (#7585) as controlled exposure (Abic-PHIBRO, Israel) at 10 weeks of age, and Reovirus Cluster II (#7585) inactivated vaccine again at 19 weeks of age based on the rationale described previously.

Detection of shedding Reovirus cluster II, was based on a specific real-time RT–PCR for reovirus cluster II. All samples were tested at the Kimron Veterinary Institute in the Laboratory of Dr. Farnoushi at the Avian diseases Department. Samples for detection of reovirus, shedding and immune response included blood samples and cloacal swabs of 25 birds from each house on the farm. Sampling started just before vaccination and then every week for 6 consecutive weeks, following sampling of blood and cloacal swabs every two weeks until the birds were moved to the breeding farms at 22 weeks of age.

The pullets were relocated at 22-23 weeks of age to three breeding farms, the fertile eggs were hatched in three commercial hatcheries and chicks were sent to different broiler farms all around the country.

Sampling for reovirus cluster II (in order to detect shedding by feces or eggs during production period) continued at the breeding farms at three sampling times: From start of lay at 26 weeks, to peak of production at 33 weeks of age. Each sampling included 25 cloacal swabs and 30 eggs from each house.

Serologic immune response after vaccination with the live Reo virus was tested using the commercial IDEXX-Avian Reovirus (REO) Ab ELISA test. Blood samples were taken before vaccination and three weeks post vaccination by controlled exposure with the live reovirus cluster II (#7585). Production parameters including mortality rates, egg production and hatchability, were recorded as routine procedure in the breeding farms from week 25 up to the end of production at 62 weeks of age.

Follow up of the progeny from the three breeding flocks, was carried out from 27 weeks to 62 weeks of age (end of production of breeding flocks). Follow up for arthritis/tenosynovitis clinical signs and condemnation rate were recorded for each broiler farm with a total follow up of more than 8

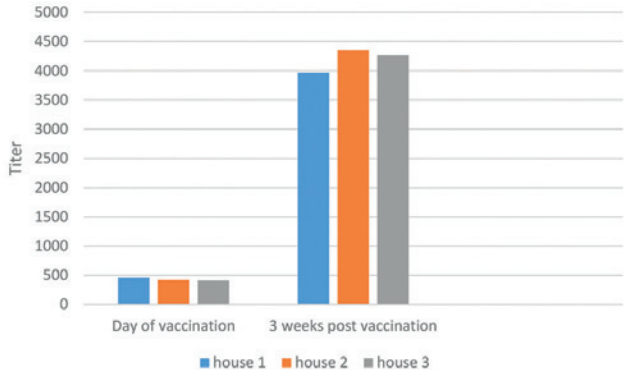


Figure 9: Immune response (ELISA reovirus antibody titers IDEXX) after vaccination with the live reovirus cluster II (#7585) by IM injection at 10 weeks of age.

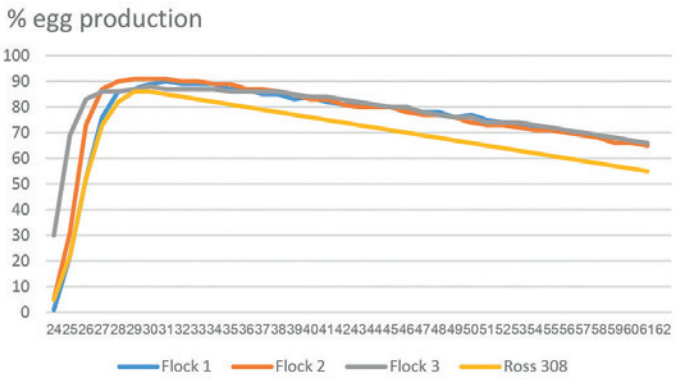


Figure10: Weekly Egg production percent of the vaccinated flocks (1, 2, 3) compared to the Ross 308 Parent stock manual handbook. 2003, p. 59-75.

million broiler chicks reared and slaughtered under commercial conditions.

RESULTS

Clinical Monitoring during the rearing period.

Individual and group clinical monitoring carried out weekly under laboratory and commercial conditions, did not reveal any adverse reactions after vaccination from day of vaccination (7 to 10 weeks of age) to week 23 in the birds exposed to IM injection of the live virulent reovirus cluster II. No development of tendinitis, joints or footpad inflammation was observed in any of the vaccinated birds reared under laboratory or commercial conditions. All birds remained healthy during the whole study and growth body weight and uniformity correlated to parameters required in the Ross 308 manual.

Serologic response to reovirus controlled exposure vaccination with the live Reo2 virus

The serologic response after vaccination under commercial conditions, revealed a good immune antibody response to reovirus measured by the ELISA commercial kit. Summary of the results are presented in Figure 9.

Viremia and Shedding of the reovirus II from the vaccinated birds under laboratory and commercial conditions.

Weekly blood and cloacal samples taken from the day of vaccination and tested by real-time RT-PCR revealed very low levels of shedding of the reovirus II. Reovirus II was detected only in cloacal samples taken between 7-14 days after vaccination (see Table 2), all the blood samples tested for viremia by real-time RT-PCR (more than 2000 samples tested) proved negative. Cloacal and eggs samples taken at time of production from the three commercial flocks collected at 23, 26 and 29 weeks of age all proved negative for the presence of reovirus by real-time RT-PCR.

Production parameters

Monitoring in all three breeding flocks proved that vaccination of the birds by controlled exposure with live reovirus II vaccination (CE adapted) did not affect any production parameter in the commercial breeding flocks and egg production. Graphs are presented in Figure 10.

Hatchability, average accumulated data in all three farms, from the start to end of production ranged from 83% to 85%, this numbers correlated with the hatchability rate according to the Ross 308 guidelines. A total of 7,783,330 broiler chicks were produced and monitored in broiler farms, only two reports of reovirus condemnation were registered out of 197 monitored flocks.

Total production data from breeding farms and chicks marketed and monitored are summarized in Table 3.

DISCUSSION

In Israel, reovirus arthritis/tenosynovitis has become in the last decade one of the most devastating economic problems in the broiler industry. Since 2007 to date, the evaluated eco-

Table 2: Monitoring results of shedding (cloacal swabs) the reovirus II before and after vaccination by controlled exposure of replacement heavy breeder pullets under laboratory and commercial conditions.

Week	Real-time RT-PCR results before and after vaccination by weekly sampling and testing								
	Pre-vacc	1	2	3	4	5	6	8	10
Laboratory Conditions	Neg	Neg	Pos (Ct-29)*	Neg	Neg	Neg	Neg	Neg	Neg
Farm 1	Neg	Neg	Pos (Ct-28)*	Neg	Neg	Neg	Neg	Neg	Neg
Farm 2	Neg	Pos (Ct-27)*	Neg	Neg	Neg	Neg	Neg	Neg	Neg

* Ct= Cycle threshold value of virus detection by real-time RT-PCR, (Ct of 30 was considered as negative for Reovirus detection). Pos= Positive real-time RT-PCR results; Neg=Negative real-time RT-PCR results.

Table 3: Total breeding production parameters and broiler monitoring results

	Breeding Hens	Eggs/Hen	Hatchability	Chicks/hen	Total No. Chicks monitored	Number of Broiler flocks monitored	Reovirus condemnation rate from flocks
Farm 1	20,159	187	85%	158	2,893,186	63	0/63
Farm 2	14,435	186	84%	156	1,851,220	64	0/64
Farm 3	19,946	185	83%	152	3,038,924	70	2/70*

* Horizontal infection 15% condemnation rate.

nomic losses in Israel due to reovirus infections were about one billion NIS (about 277 million USA Dollars). Infection of breeding flocks and egg transmission to the progeny causes severe clinical signs of tendinitis and leg deviations as early as 14 days of age, affecting between 30-80% of the birds in the flock. Most of the economic damage is due to the high condemnation rate of the affected flocks in the rearing farms and slaughterhouses. According to Jewish religious laws, affected broiler flocks suffering from inflammation or rupture of the leg tendons are considered non-Kosher thereby losing between 40-60% of their value.

Also taking into account the suffering of the birds this disease has serious welfare considerations which need to be addressed by the poultry industry.

From the four identified clusters of reovirus in Israel (8), more than 90% of the reoviruses isolated from arthritis/tenosynovitis cases in broilers belong to reovirus cluster II, which significantly differs from the reovirus cluster I, presently in current commercial vaccines. Extensive use of an autogenous inactivated reovirus cluster II vaccine in breeding flocks in Israel, helped only to reduce the shedding time of the virus to the progeny, but the economic impact of the arthritis/tenosynovitis problem remained very high.

More than 60,000 breeding birds, and almost eight million broilers (progeny) reared in almost 200 broiler flocks, were closely monitored during the study. The intensive monitoring and testing of the birds and flocks involved in the study indicated that the vaccination of the breeding flocks by controlled exposure during the rearing period (10 weeks of age), may be a safe method of vaccination against new emerging reovirus infections to the breeding flocks and their progeny.

The efficacy of the controlled exposure vaccination is now being tested under commercial conditions in Israel, with more than 1.5 million vaccinated breeding birds. Close follow up of the breeding flocks and their progeny, including the condemnation rates arthritis/tenosynovitis in the broilers at the farms, and slaughterhouses, will provide important information regarding the efficacy of this novel approach as a new tool to control reovirus infections in chickens due to Cluster II reovirus.

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