

IMMUNIZATION OF CHICKENS WITH IRRADIATED LARVAE OF *STRONGYLOIDES AVIUM*

Geraldo CHAIA (1)

SUMMARY

Thirty chickens were vaccinated, by subcutaneous route, with about 12,000 larvae of *Strongyloides avium* previously irradiated with 140 kiloroentgens. Thirty days later, both vaccinated and control chickens (8) were infected with about 1,500 normal larvae.

The mean numbers of worm recovered from vaccinated and non-vaccinated chickens (controls) were 6 (0.4%) and 262 (17.4%), respectively. The worms recovered from the former chickens displayed underdeveloped genital apparatus, no eggs having been observed in their uterus.

The chickens under experiments were thus seen to have got immunized with a single dose of the vaccine.

INTRODUCTION

Several investigators have reported the immunization of hosts by irradiated larvae of certain nematodes. JENNINGS et al.³ studied the action of X rays on the larvae of *Nippostrongylus muris*. JARRET et al.⁸ succeeded in their attempt to vaccinate calves with irradiated larvae of *Dictyocaulus viviparus*. MILLER¹² made the same experiment, on dogs, with irradiated larvae of *Ancylostoma caninum*. RADKE & SADUM¹⁴ and SZUMLEWICZ-PERLOWAGORA & OLIVER¹⁵ also studied the action of irradiation on *Schistosoma mansoni*.

CHAIA & MURTA² immunized rats with irradiated larvae of *Strongyloides (ratti + venezuelensis)*.

The present paper studies the vaccination of chickens with irradiated larvae of *Strongyloides avium*.

MATERIAL AND METHODS

In this experiments there have used thirty-eight chickens, one month old, with no helminth infection. Thirty chickens were vaccinated, by subcutaneous route, with about 12,000 larvae of *Strongyloides avium* irradiated with 140 kiloroentgens, the remaining ones (8) being taken as controls.

One month later, both vaccinated and control chickens were infected, by subcutaneous route, with about 1,500 normal larvae of *Strongyloides avium*. Eight days after infection the chickens were sacrificed and their caecum examined by MCGEE et al.¹¹ technique, the worms being counted under a dissecting microscope and examined with a microscope ($\times 160$).

Strongyloides Strain — The *Strongyloides* strain used in this experiment was from feces collected on the caecum of some chick-

Investigation carried out with financial support from the Research Council, Federal University of Minas Gerais, Brasil

(1) Research Center René Rachou — Instituto Nacional de Endemias Rurais, Caixa Postal 1.743, Belo Horizonte, Brasil

ens from the Market of Belo Horizonte. The feces were mixed with wet, granulated animal charcoal and left at 26°C for 2 days, after which filarioid larvae were removed from the coals by BAERMANN'S¹ method.

Several 0.1 ml-samples of such larvae were left, drop by drop, on a glass slide and counted under a dissecting microscope. This *Strongyloides* strain has kept in laboratory by successive infections of chickens (each 3 months).

Worm Examination — The worms were collected by the method of MCGEE et al.¹¹ and then fixed in Ble's fixative, placed in 70% alcohol with 5% glycerin added and, finally, mounted in jelly glycerin, a technique employed by LITTLE¹⁰. The worms were examined in microscope (× 160).

Irradiation of Larvae — The larvae were placed into plastic tubes in volumes, at most, 1 cm high and, then, irradiated with Gama-cell 220. This apparatus displays the following characteristics: Cobalt-60, half life-5.2 years with a dosage of 287 kilorads per hour in irradiating position, thus giving an average of 4,715 kiloroentgens per minute. The present activity of the apparatus is 3,457 curies. For gamma emissions, the roentgen is considered similar to the rad, with approximate variation of 5%.

RESULTS

The mean numbers of worms recovered from both vaccinated and control chickens were 6 (0.4%) and 262 (17.4%), respectively. The chickens that had been vaccinated showed underdeveloped genital apparatus. There has also been observed the absence of eggs in the uterus of such worms. Table I may be referred to for further data.

DISCUSSION

LEVIN & EVANS⁹ reported that *Trichinella spiralis* worms from irradiated larvae had proved to be sterile. This very same fact was observed in ancylostomiasis (dogs) by MILLER¹² and in strongyloidosis (rats) by CHAIA & MURTA².

SZUMLEWICZ-PERLOWAGORA & OLIVER¹⁵ remarked temporary paralization in the development of *Schistosoma mansoni* worms.

JARRET et al.⁷ achieved immunization in calves with irradiated larvae of *Dictyocaulus viviparus*. MILLER¹³ succeeded in vaccinating dogs with irradiated larvae of *Ancylostoma caninum*. CHAIA & MURTA², too, achieved immunization in rats by vaccinating them with irradiated larvae of *Strongyloides (venezuelensis + rattii)*.

In the present experiment the Author also observed that chickens vaccinated with irradiated larvae of *Strongyloides avium* got

TABLE I

Results obtained in chicken vaccinated with 12,000 of irradiated larvae (140 kr) and infected later on, with normal larvae of *Strongyloides avium*

Chicken used in the experiment				
Group	no. of chicken	no. of larvae infected per chicken	Worms recovered 8 days after infection	
			Mean number	Mean %
A	30	1,500	6 *	0.4
B	8	1,500	262	17.4

A — A chicken infected 30 days after having been vaccinated

B — Control chicken

* — Most worms were immature

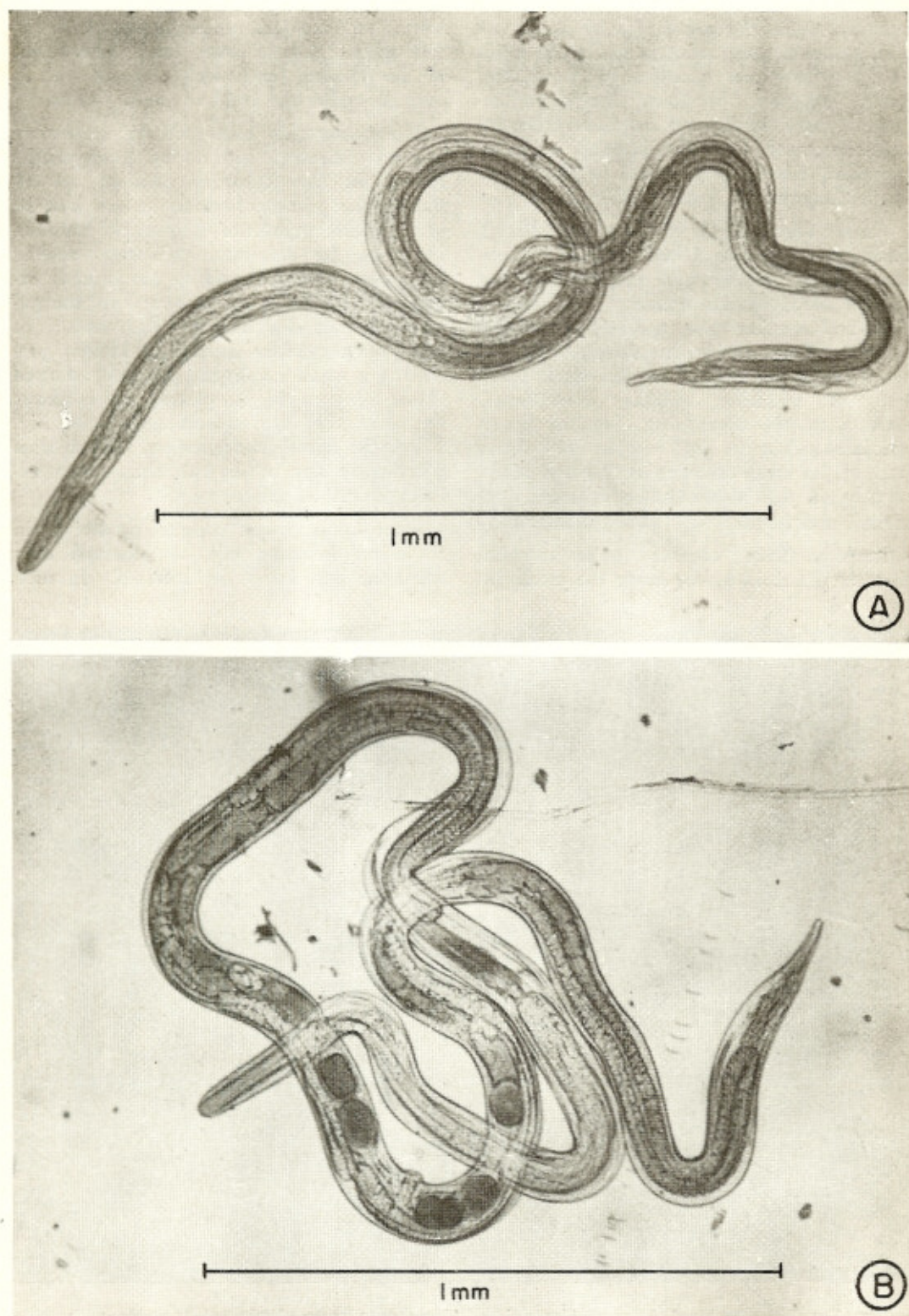


Fig. 1 — Parthenogenetic females of *Strongyloides avium*. A) Worm from vaccinated chicken; B) Normal worm

immune, since the percentage of worms recovered from these animals was much lower (0.4%) than that obtained in the control group (17.4%). Moreover, the worms recovered from vaccinated chickens presented underdeveloped genital apparatus, no eggs, therefore, having been found in the uterus of parthenogenetic females (Fig. 1, A).

CRAM³ observed, for the first time (1929), *Strongyloides* worms in the caecum of chickens (*Gallus gallus*) and named this new species *Strongyloides avium*. TRAVASSOS¹⁷ described another new species of strongyloides — *Strongyloides oswaldoei* — that he observed to be present in the small intestine of chickens (*Gallus domesticus*). CRAM⁴, in the same year, detected *Strongyloides avium* in the caecum and lower part of the small intestine of 2 white birds, namely, in the junco (*Junco hyemalis hyemalis*) and in the coot (*Fulica americana*).

Later on CRAM⁵ observed, to his surprise, that the filarioid larvae from the small in-

testine of chickens when employed to infect other chickens were found, afterwards, to be located, exclusively, on the birds' caecum under the form of adult worms.

We are strongly convinced the species we are experimenting with to be *Strongyloides avium* for the following reasons: a) although the parasite location on the host be of no great importance, it is worth mentioning that the specimens we were dealing with were always found to be located, exclusively, on the host's caecum; b) the parasitic female described by TRAVASSOS¹⁷ as being *Strongyloides oswaldoei*, besides presenting a small esophagus, generally showed about 50 eggs in its uterus, which shows the species we are experimenting with Fig. 1, B to be *Strongyloides avium*, since it does not display any of the two chief characteristics described above.

Some pathological aspects occurring in *S. avium* infection can be observed only when the host is heavily infected. In such



Fig. 2 — Eggs of *Strongyloides avium*. These eggs were eliminated together with fragments of intestinal mucosa

a case the host very frequently ejects diarrheic feces with a lot of mucus and blood, the eggs being eliminated together with fragments of the intestinal mucosa (Fig. 2).

Although *S. avium* does not prove to be such a problem as to discourage the breeding of chickens, since in modern coops the birds are hardly in contact with the soil, nematode infection being then avoidable, we find it interesting to immunize the chickens against such infection by using a vaccine from irradiated *Strongyloides avium*.

RESUMO

Imunização de pintos com larvas irradiadas de *Strongyloides avium*

Trinta pintos foram vacinados por via subcutânea, com uma média de 12.000 larvas de *Strongyloides avium*, as quais foram irradiadas com 140 kiloroentgens. Trinta dias após a vacinação, os pintos vacinados e os controles foram infetados com uma média de 1.500 larvas normais.

Os números médios de vermes recuperados nos pintos vacinados e nos controles foram, respectivamente, de 6 (0,4%) e de 262 (17,4%). Os vermes provenientes dos pintos vacinados não apresentaram com o aparelho genital desenvolvido e, conseqüentemente, não foi observada a presença de ovos no útero.

Os pintos ficaram imunizados apenas com uma única dose da vacina.

REFERENCES

1. BAERMANN, G. — Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Mededel. mit. h. Geneesk. Lat. te Weltwreden — Feestbundel, Batavia, pp. 41-47, 1917.
2. CHAIA, G. & MURTA, C. C. — Immunization of rats with irradiated *Strongyloides* larvae. *Rev. Inst. Med. trop. São Paulo* 9:163-168, 1967.
3. CRAM, E. B. — A new roundworm parasite, *Strongyloides avium* of the chicken, with observations on its life history and pathogenicity. *North Amer. Vet.* 19:27-30, 1929.
4. CRAM, E. B. — New host records for *Strongyloides avium*. *J. Parasit.* (Proc. Helm. Soc. Wash.) 17:55-56, 1930.
5. CRAM, E. B. — Biological and morphological observations on a species of *Strongyloides* (Nematoda) of chickens in Puerto Rico. *Rev. Parasit. Clin. Lab.* 2:289-304, 1936.
6. JARRET, W. F. H.; JENNINGS, F. W.; McINTYRE, W. I. M.; MULLIGAN, W. & URQUART, G. M. — Immunological studies on *Dictyocaulus viviparus* infection. Immunity produced by the administration of irradiated larvae. *Immunology* 3:145-151, 1960.
7. JARRET, W. F. H.; JENNINGS, F. W.; McINTYRE, W. I. M.; MULLIGAN, W. & URQUART, G. M. — Immunological studies on *Dictyocaulus viviparus* infection. Active immunization with whole worms vaccine. *Immunology* 3:135-144, 1960.
8. JENNINGS, F. W.; MULLIGAN, W. & URQUART, G. M. — Variables in X-Ray "Inactivation" of *Nippostrongylus brasiliensis* larvae. *Exp. Parasit.* 13:367-373, 1963.
9. LEVIN, A. J. & EVANS, T. C. — The use of roentgen radiation in locating an origin of host resistance to *Trichinella spiralis* infection. *J. Parasit.* 28:477-483, 1942.
10. LITTLE, M. D. — Comparative morphology of six species of *Strongyloides* (Nematoda) and redefinition of the genus. *J. Parasit.* 52:69-84, 1966.
11. MCGEE, G.; RUSSOMANO, R. & SANDGROUND, J. H. — An expeditions method for counting trichostrongylids and other helminths of the small intestine. *J. Parasit.* 43:315-317, 1957.
12. MILLER, T. A. — Comparison of the immunogenic efficiencies of normal and X-irradiated *Ancylostoma caninum* larvae in dogs. *J. Parasit.* 52:512-519, 1966.
13. MILLER, T. A. — Effect of X-Irradiation upon the infective larvae of *Ancylostoma caninum* and the immunogenic effect in dogs of a single infection with 40 kr-irradiated larvae. *J. Parasit.* 50:735-742, 1964.
14. RADKE, M. G. & SADUN, E. H. — Resistance produced in mice by exposure to irradiated *Schistosoma mansoni* cercarie. *Exp. Parasit.* 13:134-142, 1963.
15. SZUMLEWICZ-PERLOWAGORA, A. & OLIVER, J. L. — *Schistosoma mansoni*: Development of challenge infections in mice

- exposed to irradiated cercariae. *Science* 140:411-412, 1963.
16. SZUMLEWICZ-PERLOWAGORA, A. — Studies on acquired resistance to *Schistosoma mansoni* in mice exposed to X-irradiated cercariae. *Bull. Org. Mond. Santé* 30:401-412, 1964.
17. TRAVASSOS, L. — Pesquisas helmintológicas realizadas em Hamburgo. VII — Notas sobre os Rhabdiasoidea Railliet, 1916. *Mem. Inst. Oswaldo Cruz* 24:161-181, 1930.

Recebido para publicação em 20/8/1968.