

Egg yolk IgY antibodies against antigens of the hookworm *Ancylostoma ceylanicum*

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Hookworm infection is considered a neglected tropical disease and is caused by nematodes from the Ancylostomatidae family. Approximately 740 million people worldwide are infected with this parasite. Specific antibodies have been used to detect helminth antigens on faeces and tissue samples from infected hosts or used to select antigenic targets. Avian egg yolk antibodies, named IgY, are alternative to IgG as follows: (i) immunized laying hens transfer IgY antibodies from blood to egg yolk; (ii) high-avidity IgY antibodies are purified from egg yolk by a low-cost process avoiding animal bleeding; (iii) IgY antibodies do not interact with rheumatoid factor, mammalian complement protein or Fc receptor. This study aimed to produce and purify specific IgY antibodies against *Ancylostoma ceylanicum* larvae. The saline extract (SE) from *A. ceylanicum* filarial larvae was evaluated by 12% polyacrylamide gel electrophoresis (SDS-PAGE) to characterize the protein profile, and subsequently used to immunize hens for obtainment of specific IgY antibodies. Four 25-week-old laying hens (*Gallus gallus domesticus*, Hisex lineage) were immunized in the pectoral muscle with *A. ceylanicum* SE using Freund's adjuvant. The immunization protocol was carried out four times at 14-day intervals using 100 µg of SE diluted in 250 µL of phosphate-buffered saline (PBS 0.01M, pH 7.2) and 250 µL of complete or incomplete Freund's adjuvant. Egg yolks were collected, pooled and purified weekly. IgY antibody purification was confirmed by 12% SDS-PAGE. The specificity of the produced antibodies was confirmed by immunoblotting, in which the bands of 250, 190, 44 and 42 kDa were recognized. ELISA was used to perform the kinetics of anti-*A. ceylanicum* larvae IgY production in serum and purified egg yolks. The production of specific antibodies presented detectable levels of specific IgY in the weeks 6, 8 and 14 after immunization. The avidity index (AI) was 59% in the week 5 after immunization, there was a reduction to 42% at the 6th week and the highest AI value (61%) was reached in the 10th week. Specific IgY antibodies against *A. ceylanicum* larvae were produced and purified, highlighting this method as economically feasible and reproducible with high antibody yield.

Keywords: avian antibodies, helminth, egg yolk, IgY, hookworm.

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