

KINETICS OF ANTIBODY RESPONSE BY DOT-ELISA IN RABBITS IMMUNIZED WITH ADULT *HAEMONCHUS CONTORTUS* ANTIGEN

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Summary:

Whole adult soluble extract of *Haemonchus contortus* as an antigen along with Freund's complete adjuvant, was used to immunize rabbits. Antisera from immunized rabbits were collected at intervals of 30, 60, 90, 120, 150 and 180 days. For the detection and titration of anti-*H. contortus* antibodies in these sera, Dot-ELISA was developed. Sera collected 30 days post-immunization exhibited a titre of 1:5,000 in all the rabbits except one, where a titre of 20,000 was recorded. Later, all the rabbits attained the highest titre of 40,000 at different periods of post-immunizations, which were maintained 150-180 days. These high titre sera can be of immense use in the identification and characterization of immunodominant antigens of adult *H. contortus*.

KEY WORDS : *Haemonchus contortus*, Dot-ELISA, antigen, immune response.

Résumé : ÉTUDE PAR DOT-ELISA DE LA RÉPONSE IMMUNITAIRE DE LAPINS IMMUNISÉS PAR DES ANTIGÈNES D'*HAEMONCHUS CONTORTUS* ADULTE

Un extrait soluble d'adulte d'*Haemonchus contortus* associé à l'adjuvant de Freund a été utilisé en tant qu'antigène pour immuniser des lapins. Les antisérums des lapins ont été prélevés aux 30, 60, 90, 120, 150 et 180^{èmes} jours après immunisation. La détection et le titrage des anticorps anti *H. contortus* ont été faits par la technique Dot-ELISA. Les sérums prélevés aux 30^{ème} jour ont montré un titre de 1/5000 chez tous les lapins, sauf un pour qui le titre était de 1/20000. Plus tard, tous les lapins atteignent le titre de 1/40000 qui se maintient jusqu'aux 150-180^{èmes} jours. Ce titre élevé d'anticorps sériques pourrait être d'un grand intérêt dans l'identification et la caractérisation des antigènes d'*H. contortus* adulte.

MOTS CLÉS : *Haemonchus contortus*, Dot-ELISA, antigène, réponse immunitaire.

Haemonchus contortus is a blood-sucking nematode parasite primarily occurring in the abomasum of ruminants, notably sheep and goats. It has been ranked as the most important parasite of small ruminants in all regions across tropics/sub-tropics (Anon, 1992). Haemonchosis, the disease caused by this nematode is responsible for considerable economic losses worldwide. In hyperacute disease, death of the host may occur within one week without significant signs (Fraser, 1991). In view of the severe pathogenicity and economic losses due to this parasite, various control strategies are being studied e.g. chemotherapy, breed resistance, biological control and immunological control through vaccines.

In immunological control methods, with vaccine as target, specific immunodominant antigens play a significant role. Kinetic studies of immune sera can be of significant help in the titration studies as well as recognition of antigenic components of protective value. Keeping this in viewpoint, Dot-ELISA was used to study the kinetics of hyperimmune sera of rabbits immunized with adult *H. contortus* antigen.

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MATERIALS AND METHODS

Live adults (both male and female) of *H. contortus* were collected from the abomasa of goats (*Capra hircus*), procured from local abattoirs. The collected parasites were cleaned by thorough washing in normal saline and then in 0.05M PBS, pH = 7.2. These were then stored in 0.2M Tris-HCl buffer, pH = 8.2, at – 20° C till further use.

ANTIGEN PREPARATION

The collected parasites were homogenized in Tris-HCl buffer for five minutes and sonicated at 7 μ for eight minutes. The homogenate was centrifuged at 10,000 rpm for 20 min at 4° C. The supernatant obtained was used as antigen. The protein content of antigen was determined by the method of Lowry *et al.*, 1951.

ANTIBODY PRODUCTION

The outbred rabbits kept in the laboratory were acclimatized in the laboratory for 15 days before the start of experimentation. They were immunized with *H. contortus* antigen along with Freund's complete adjuvant during first immunization and with Freund's incomplete adjuvant during second immunization after 30 days. This was followed by monthly booster immu-

nizations without adjuvant for 180 days. The route of immunization followed was intradermal for first immunization and intramuscular for subsequent booster immunizations. 400 µg of antigen was used per rabbit. Before starting the experiment, the animals were bled to collect preimmune sera. The animals were then bled at regular intervals of 30, 60, 90, 120, 150 and 180 days for collection of sera followed by booster immunizations.

DETECTION AND TITRATION OF ANTIBODIES

Indirect Dot-ELISA was used for the detection and titration of anti- *H. contortus* antibodies for the kinetic studies. The test antigen diluted to different concentrations (10 µg/µl – 1 ng/µl) in PBS pH = 7.2 was coated in the form of dot in the centre of NCM pad of comb-shaped dipsticks and incubated for overnight at 4°C. Blocking buffer used was 3 % lactogen in PBS. Antisera from rabbits were serially diluted to 1:100 – 1:80,000 in PBS and dispensed in 96-well microtitre plate followed by incubation at 37°C for one hour. The dipsticks were then washed twice with PBS, followed by incubation with goat-anti rabbit HRPO conjugate for one hour at 37°C. The combs were then given four washings with PBS and dipped in substrate solution (5 mg 3,3'-Diaminobenzidine hydrochloride/10 ml PBS + 10 µl of 0.06 % H₂O₂) for 5-15 min. The reaction was stopped by dipping the combs in distilled water. Positive test appeared as a dark-brown dot against white back ground.

RESULTS

Indirect Dot-ELISA revealed coloured spot in sera dilution up to 1:40,000 or 1:40 K and no color in dilution 1:80 K (Fig. 1).

The maximum titre values of sera of all experimental rabbits collected at different intervals before booster immunizations are shown in Table I.

Days	Titres of anti- <i>Haemonchus contortus</i> sera (in K) by Dot-ELISA					
	R-1	R-2	R-3	R-4	R-5	R-6
30	20	5	5	5	5	5
60	40	20	20	10	40	40
90	40	40	40	10	20	10
120	40	40	10	5	5	10
150	40	40	20	40	20	20
180	20	40	5	20	20	20

R represents Rabbit.

K represents 1,000.

Table I. – Kinetics of anti-*Haemonchus contortus* antibodies by Dot-ELISA.

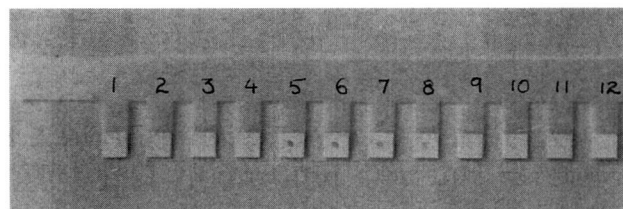


Fig. 1. – Titration of anti-*Haemonchus contortus* antibodies by Dot-ELISA.

Spot	1. No antigen, no antibody	antigen = 1 µg/µl
	2. No antigen, immune rabbit serum (1:100)	
	3. No antibody	
	4. Pre immune serum - 1:100	
	5. Immune rabbit serum - 1:500	
	6. Immune rabbit serum - 1:1,000 or 1:1K	
	7. Immune rabbit serum - 1:2K	
	8. Immune rabbit serum - 1:5K	
	9. Immune rabbit serum - 1:10K	
	10. Immune rabbit serum - 1:20K	
	11. Immune rabbit serum - 1:40K	
	12. Immune rabbit serum - 1:80K	

In rabbit 1, the initial titre after 30 days was 20 K which remained steady at 40 K till 150 days and then decreased to 20 K at 180 days.

In rabbits 2 and 3, the initial titre of 5 K increased to 40 K after 90 days in both cases and was maintained till 180 days in case of rabbit 2 but decreased to 5 K in rabbit 3.

In case of rabbit 4, the highest titre of 40 K was revealed after 150 days and decreased to 20 K at 180 days.

In rabbits 5 and 6, a titre of 40 K was reached after 60 days, but decreased to 20 K after 180 days.

DISCUSSION

Antibody levels of sera collected at intervals and checked by Dot-ELISA showed that adult anti-*H. contortus* antibody levels were maintained till the end of the experiment i.e. 150 and 180 days (Table I) which may be due to secondary immune response stimulated by booster immunizations. A constant increase in antibody level against *H. contortus* excretory/secretory (E/S) antigens was observed in sheep, from two weeks after first injection and each subsequent vaccine injection boosted humoral immune response (Schallig & Leeuwen, 1997). Even serum antibody response of Texel sheep experimentally infected with larvae suggested an immunological memory for *H. contortus* larval antigens (Schallig *et al.*, 1994). Results revealed an individual variation in immune responses to *H. contortus* antigens in immunized rabbits. Similar results were obtained in random-bred sheep immunized with H-gal-GP (*Haemonchus* galactose containing glycoprotein) complex (Smith *et al.*, 1999) and in vaccination studies with E/S antigens of adult

H. contortus of MW 15 and 24kDa (Schallig & Leeuwen, 1997). This is most likely to be caused by genetically determined variation in the ability of individuals to respond to parasites (Dineen *et al.*, 1978; Gray, 1987). The rabbits used in the present studies were outbred which might explain for individual variation in immune response.

The kinetics of anti-*H. contortus* antibody responses in serum and faecal extracts of natural host i.e. sheep, experimentally infected with *H. contortus* have been studied previously using iso-type specific ELISA (Gill *et al.*, 1993). In comparison to somatic antigens, early maximal antibody response to E/S antigens was observed in studies on kinetics of antibody response against third stage larvae of *Anisakis simplex* by ELISA (Iglesias *et al.*, 1993).

In the present studies, a high titre sera were obtained in immunized rabbits and observed to be maintained for 180 days, in response to adult *H. contortus* soluble extract which can be of significant value in the identification of immunodominant antigens and their further characterization, using natural infected hosts sera.

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Reçu le 5 mars 2002

Accepté le 16 juillet 2002

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