National Research Center

Induction of protective cellular and humoral responses against fasciolosis in rabbits using immunoaffinty fraction of Fasciola gigantica excretory secretory products

By

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Fasciolosis

Fasciolosis is a worldwide zoonotic disease caused by trematode parasite of the genus *Fasciola*.

WHO (2011) estimates that at least 2.4 million people are infected in more than 70 countries world wide, with several million people at risk.

Recently, *Fasciola sp.* was added to the WHO list of neglected tropical diseases after decades of neglect (WHO, 2010).

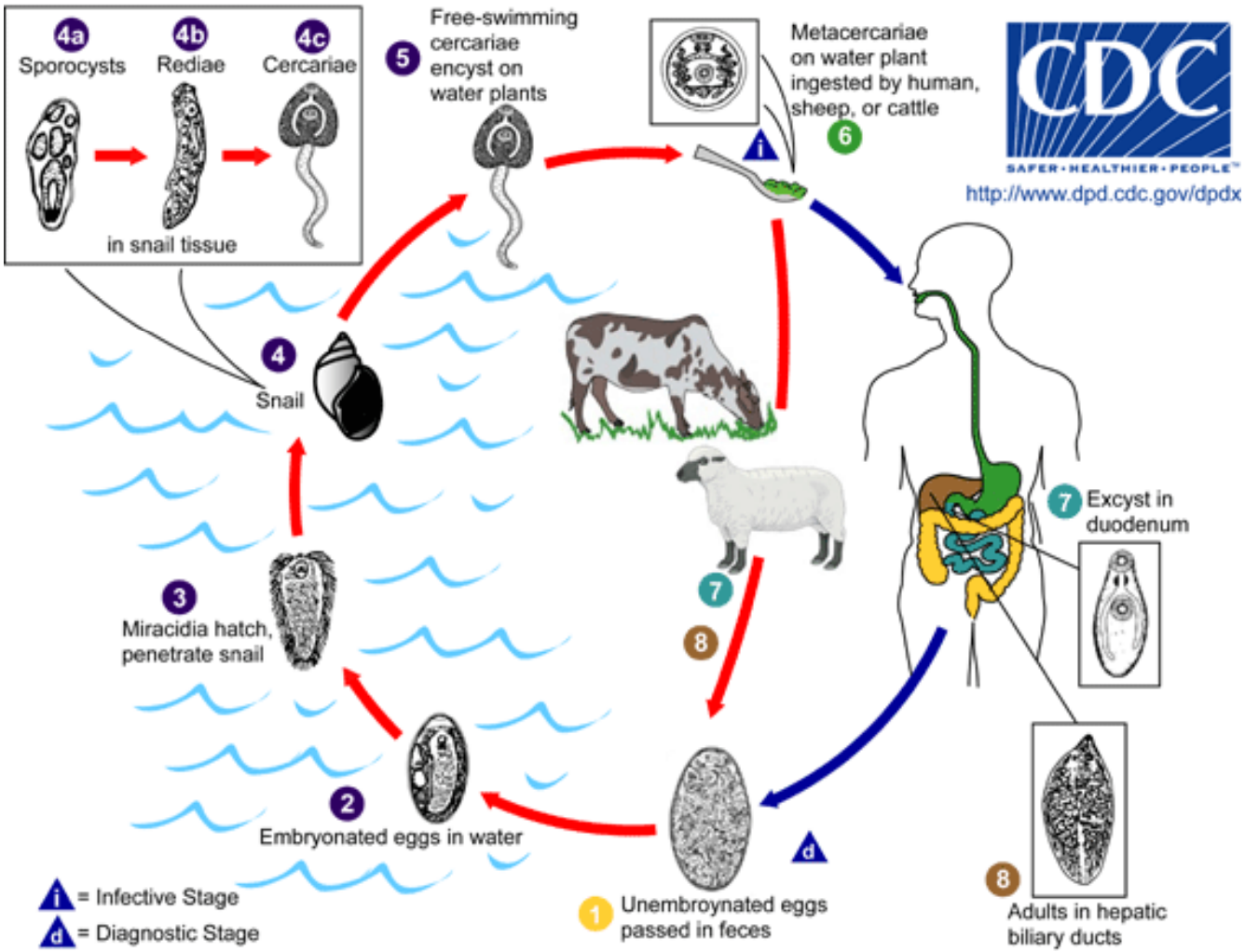
Fasciolosis

- **No continent is free from fasciolosis, and it is likely that where animal cases are reported, human cases also exist (WHO, 2011).**

Economic losses

Fasciola causes huge economic losses of over 3 billion $ Dollars to livestock production and food industry

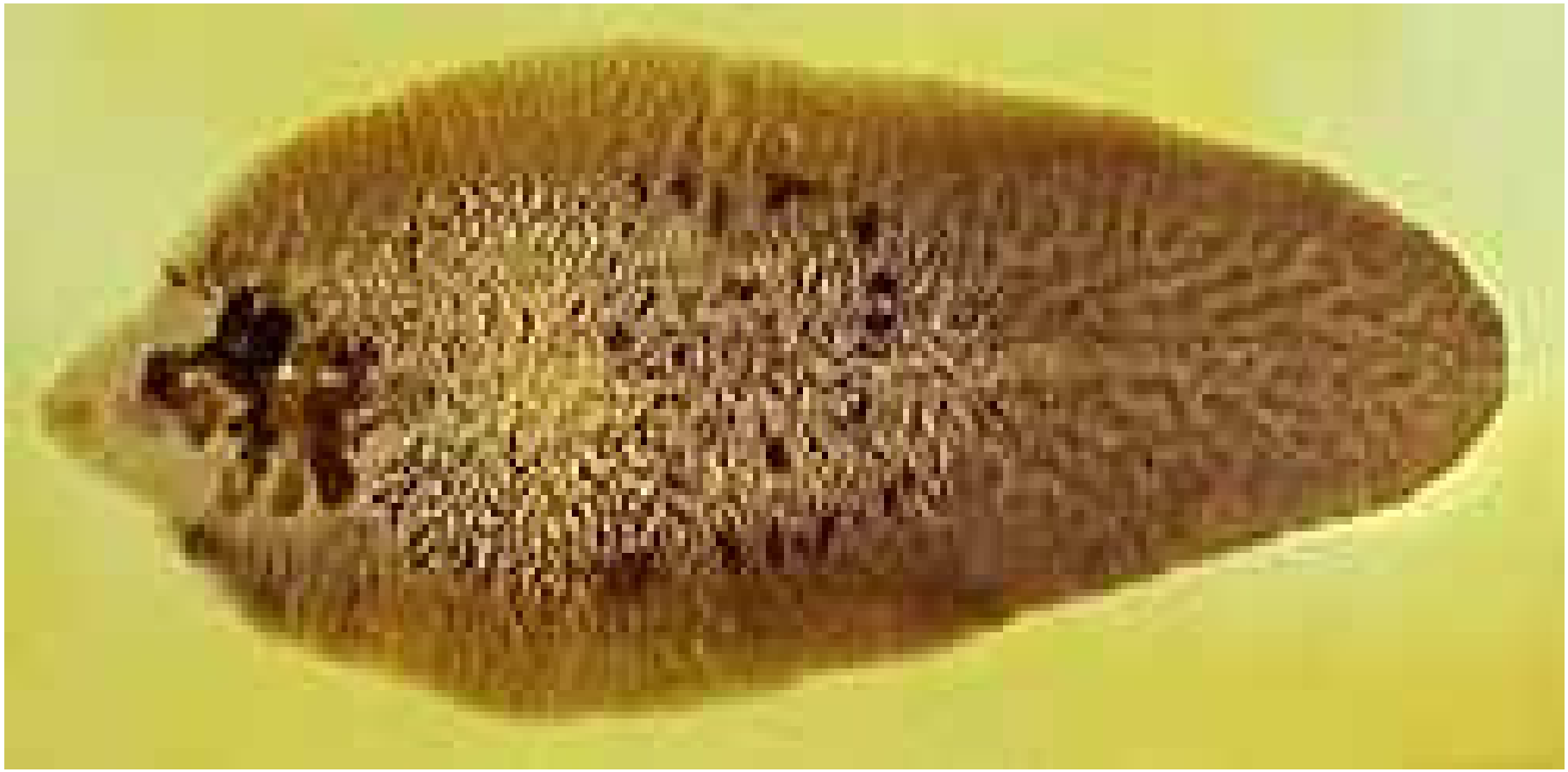
Fasciola life cycle



Treatment

- **Triclabendazole is still the most effective drug for combating the disease.**
- **Chemotherapy of fasciolosis is expensive, less effective along with development of drug resistance**

Fasciola worm



Liver Fluke

- The liver fluke secretes molecules, known as excretory-secretory (ES) products that modulate or suppress host immune responses.
- These molecules include some enzymes and fatty acid binding proteins (FABP)
- They have the potency of inducing a protective response against *Fasciola* in laboratory animals and large animal models

Vaccine candidates

- **Many candidate proteins have been tested for a long time as**
- **Fatty acid-binding proteins,**
- **Glutathione S-transferases,**
- **Cathepsin proteases,**
- **Leucine aminopeptidase,**
- **Fluke haemoglobin and**
- **Thioredoxin Peroxidase.**

Immune response

Host immune response includes Th1 cells which produce many cytokines, including IFN-γ and TNF-α, and promote the activation of macrophages which lead to the production of opsonizing antibodies.

Th2 cells produce many other cytokines, including IL-4, IL-6, and IL-10.

Helmenthiasis response

Generally, helminth infections are manifested by suppression of Th1 function and induction of T cells, which express cytokines characteristic of the Th2 subset.

No commercial vaccines

- **No commercial vaccine is currently available.**

No commercial vaccine

- **Many factors may be responsible for the failure of tested vaccines as**
- **vaccine formulation,**
- **choice of adjuvant and route of delivery and dosage**
- **and, possibly, the choice of target antigen.**

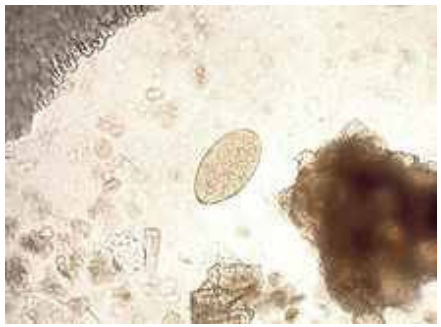
Objective

- **Thus developing vaccines for controlling animal and human fasciolosis is priority.**

Identification of new target antigens for developing an effective vaccine against fasciolosis is a hot area of research

Materials and Methods

a- Rabbits



- Twenty five native breed
- rabbits (1.25 – 1.5 Kg)
- were used.
- Faecal samples of each rabbit were examined microscopically in the laboratory for *Fasciola* eggs and they were found free from *Fasciola* and other parasitic infections.

b- Buffaloes

- A total 134 blood samples and their corresponding faecal samples were individually obtained from buffaloes in the abattoir. Moreover, gall bladders and livers were collected for post mortem examinations

Rabbits as experimental model

Rabbits are used as models in different studies as

- Evaluation of novel antibiotic formulations as therapy against bacterial or parasitic infections
- production of high quality antiserum, in studies of immunoglobulin structure and regulation
- **vaccination studies against parasites and viral infections**

Materials and Methods

- **Parasites**
- **a- Adult *Fasciola gigantica* worms:**
- Adult *Fasciola* worms were collected from condemend livers naturally infected with fasciolosis from buffaloes slaughtered in Cairo abattoir.
- **b- Metacercariae**
- *Fasciola gigantica* encysted metacercariae were purchased from Theodor Bilharz Research Institute, Egypt.

Materials and Methods

Preparation of adult *F. gigantica* excretory-secretory antigen (FgESPs)

After washing living mature *F.gigantica* were maintained in RPMI – 1640 pH 7.3, containing 2% glucose and 25 mg/L gentamicin at 37ºC overnight.

Materials and Methods

Rabbit hyperimmune serum

- About 40 µg/Kg of *F. gigantica* ESPs was mixed with of Freund's complete adjuvant and injected subcutaneously into each of 5 rabbits
- A booster dose of ESPs in Freund's incomplete adjuvant was injected two weeks later Second and third booster doses were given on days 21 and 28.

Materials and Methods

Affinity purification of adult *F. gigantica* ESPs antigen:

- Crude ESPs was applied to the column composed of CNBr –Sepharose 4B coupled with anti- ESPs
- The bound material was eluted with 50 mM glycine – 500 mM Nacl – 0.02 % w/vNaN3 PH 2.3.

Materials and Methods

SDS- PAGE

Reducing conditions

10% slab gel

Silver stain

Vaccination protocol

Three groups

1- Normal group injected with buffer

2- Control infected

3- Vaccinated challenged group

Vaccination protocol

Dose: 40µg/Kg

Route: subcutaneous

Times: twice

Adjuvant: Freund's (Complete and incomplete)

Challenge: 30 metacercariae orally

Blood samples: before immunization untile 10 weeks post challenge

Assessment of protection

post mortem examination of animals

- **a- Fluke recovery**
- **b-Fluke size**

Assessment of Diagnostic & Protective value

Humoral response (IgG levels)

ELISA

Coating: 5µg/ml

Serum samples: Rabbits&Buffaloes(1:100)

Secondary Antibody: Anti-buffaloe and rabbit IgG horse radish peroxidase labeled-conjugates (1:1000)

Substrate: ortho-phenylenediamine (OPD)

Assessment of Protective value

Cellular Response

Levels of IL-4

INFγ

ELISA

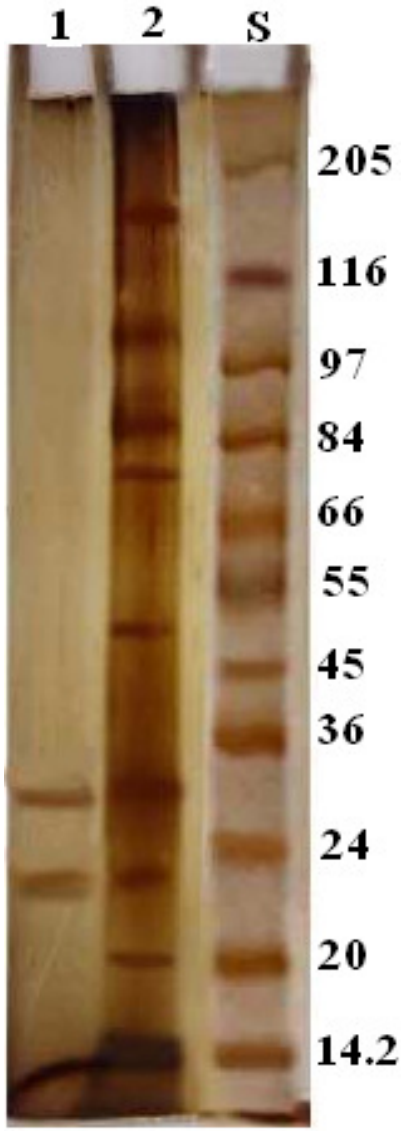
Results
Purification

Fraction	Total protein (µg x 104)	Activity unit Au x 106	Specific activity (Au/µg x102)	Purification fold	Yield (%)
Crude ES	29.2	7.3	0.25	1.00	100.00
Unbound fraction	**16.3**	0.5	0.031	0.22	6.85
Bound fraction	**0.19**	6.4	33.53	**2051.5**	**87.67**

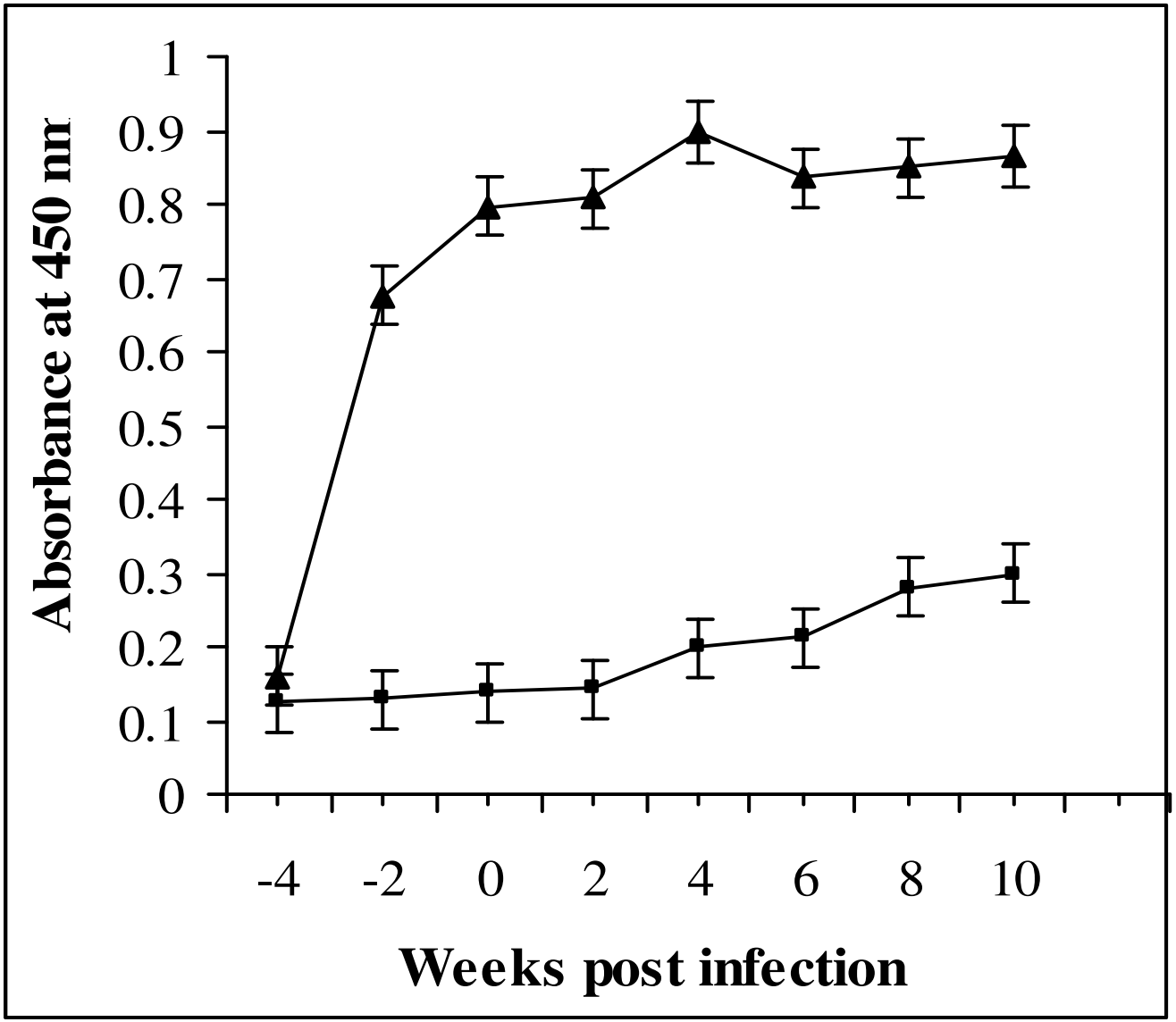
Parasitological Evaluation

Groups	**Worm recoveries 10 weeks after challenge Mean ± SD**	**Worm burden reduction**	**Worm Size (mm) Mean ± SD**
Group1	**20**.0±1.019	-	**21**±0.16
Group2	**3**± 1.04	**85 %**	**10.4**±0.15

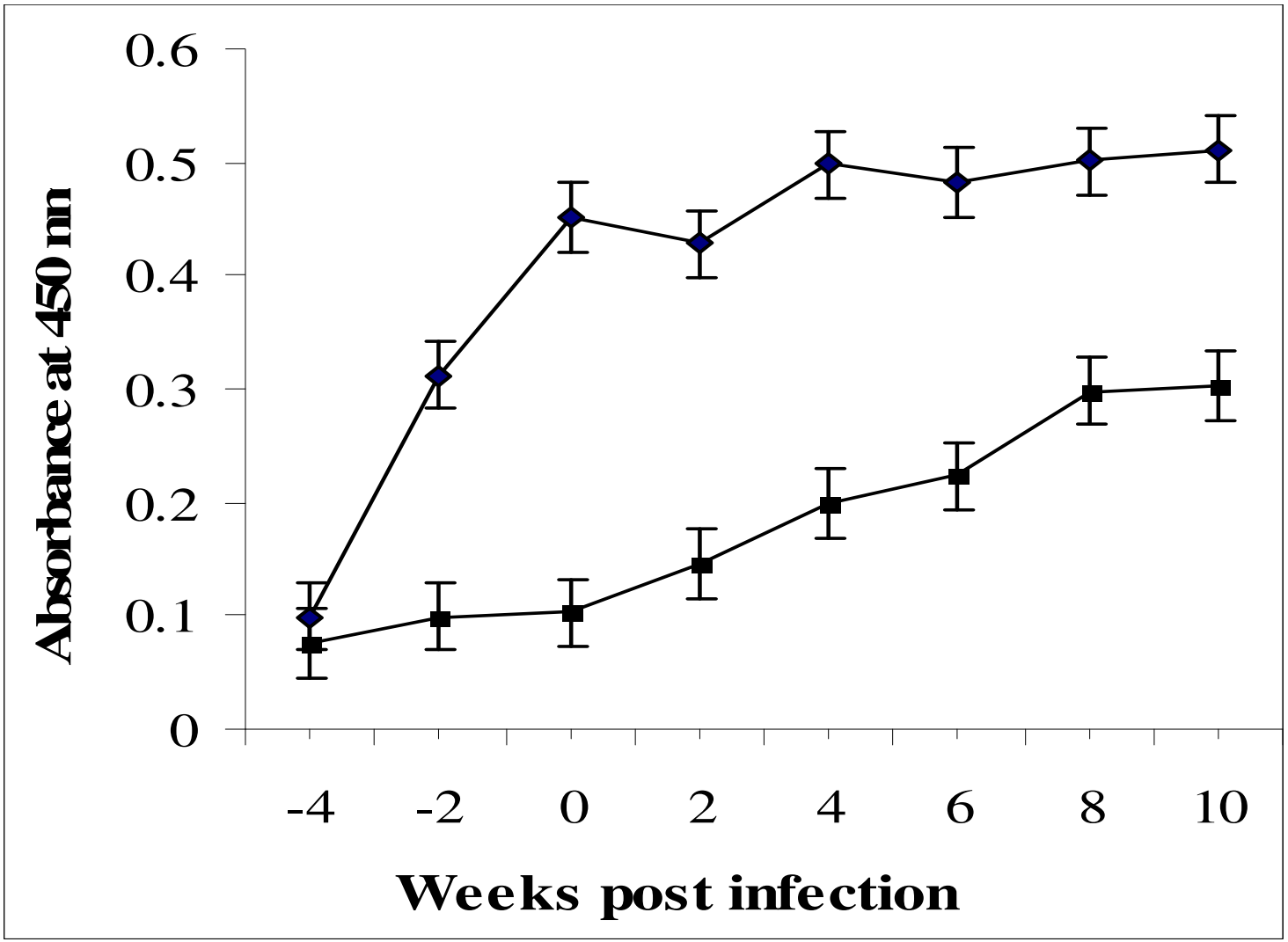
Electrophoretic profile



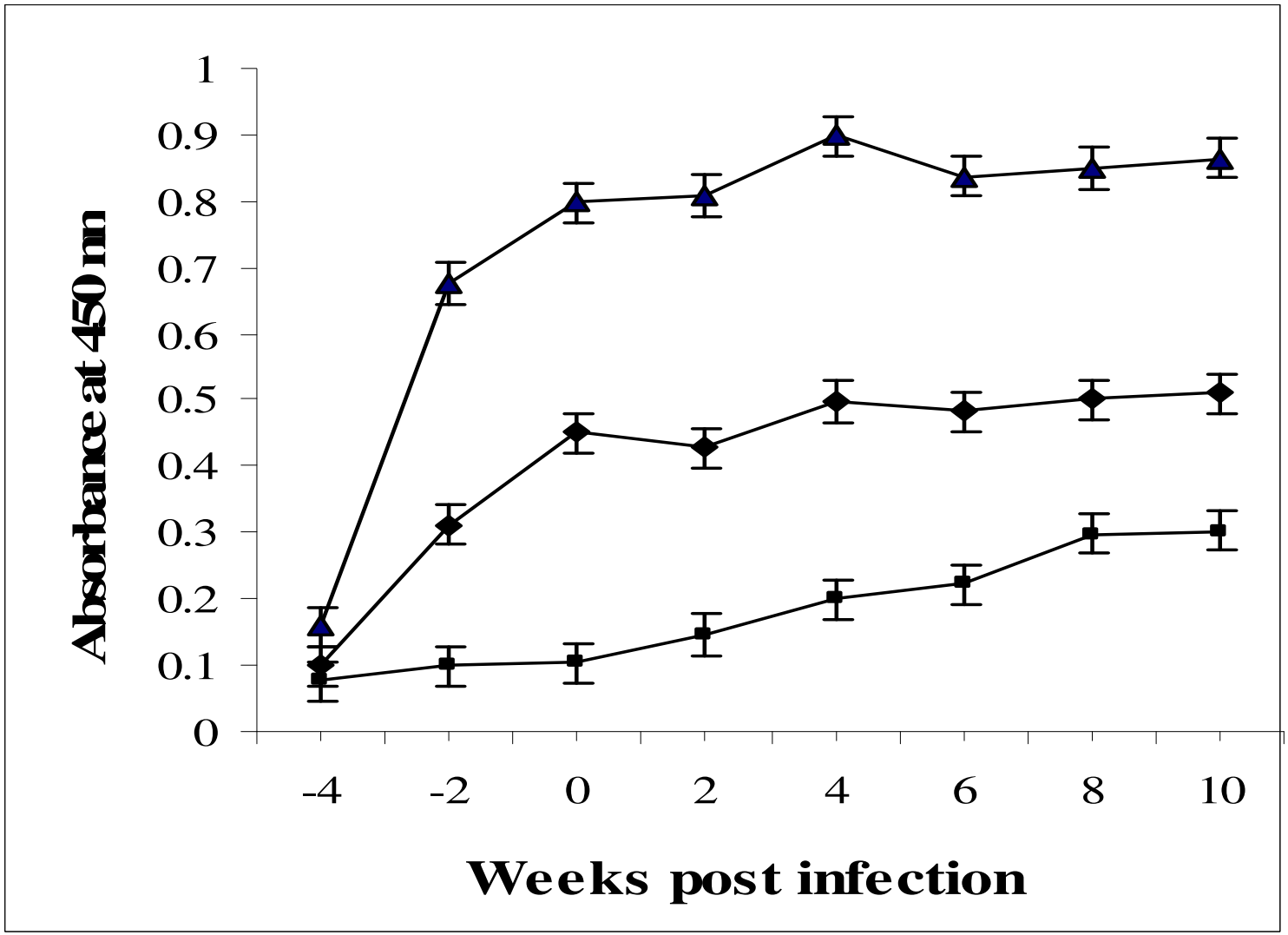
Protective Rabbit IgG antibody response to the fraction



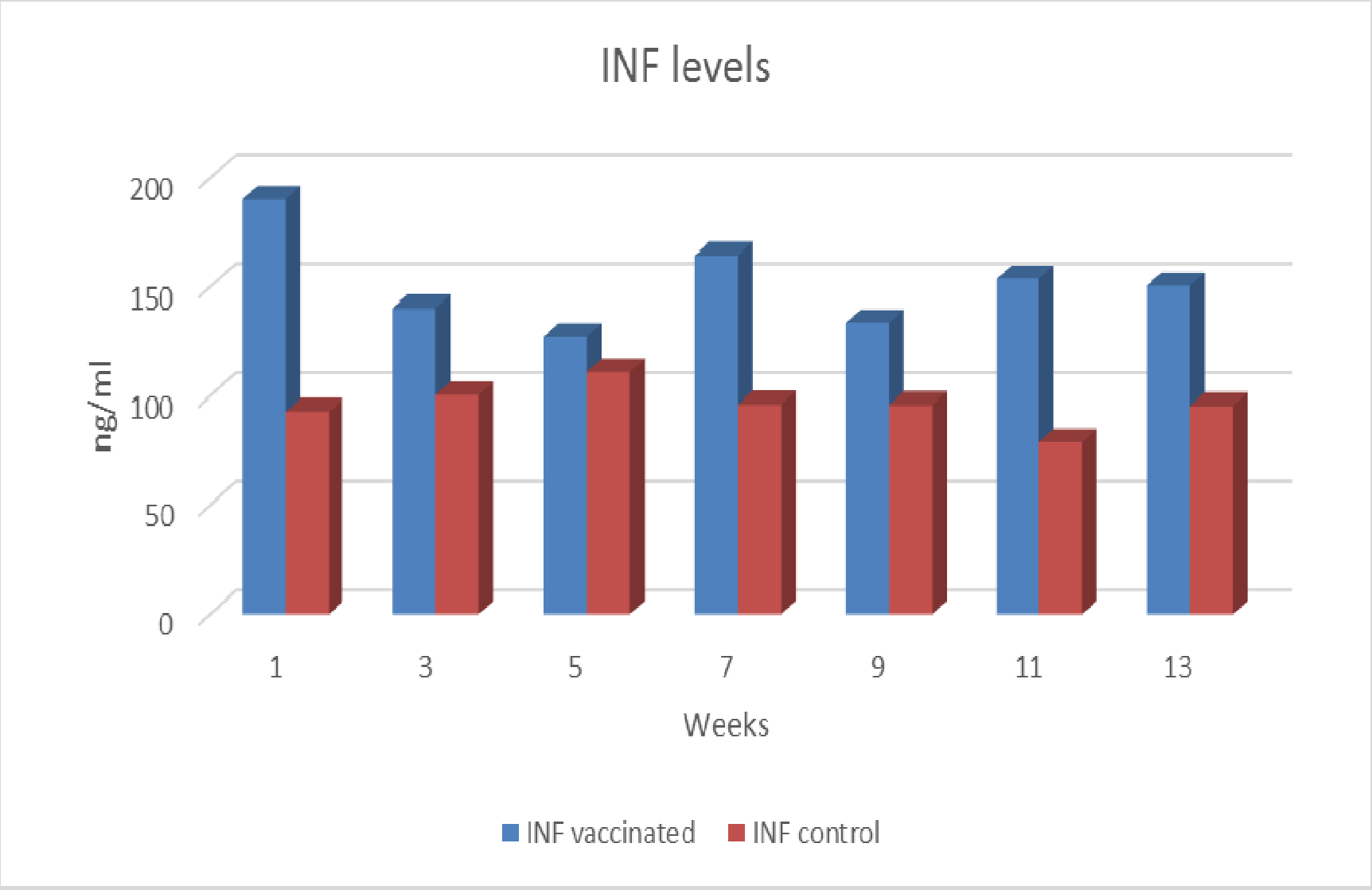
Protective Rabbit IgG antibody response to crude extract



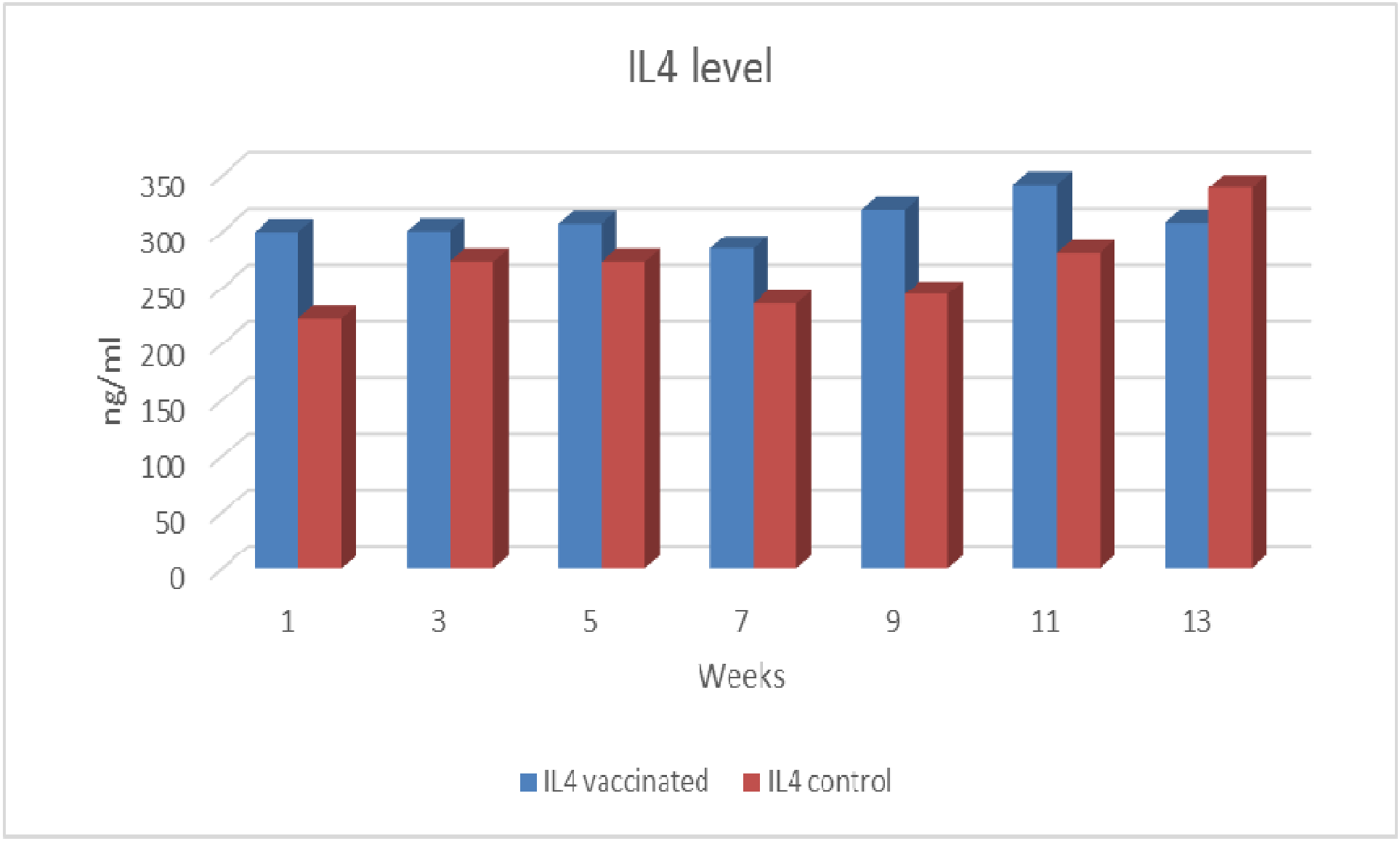
IgG antibody response to the fraction and crude extract



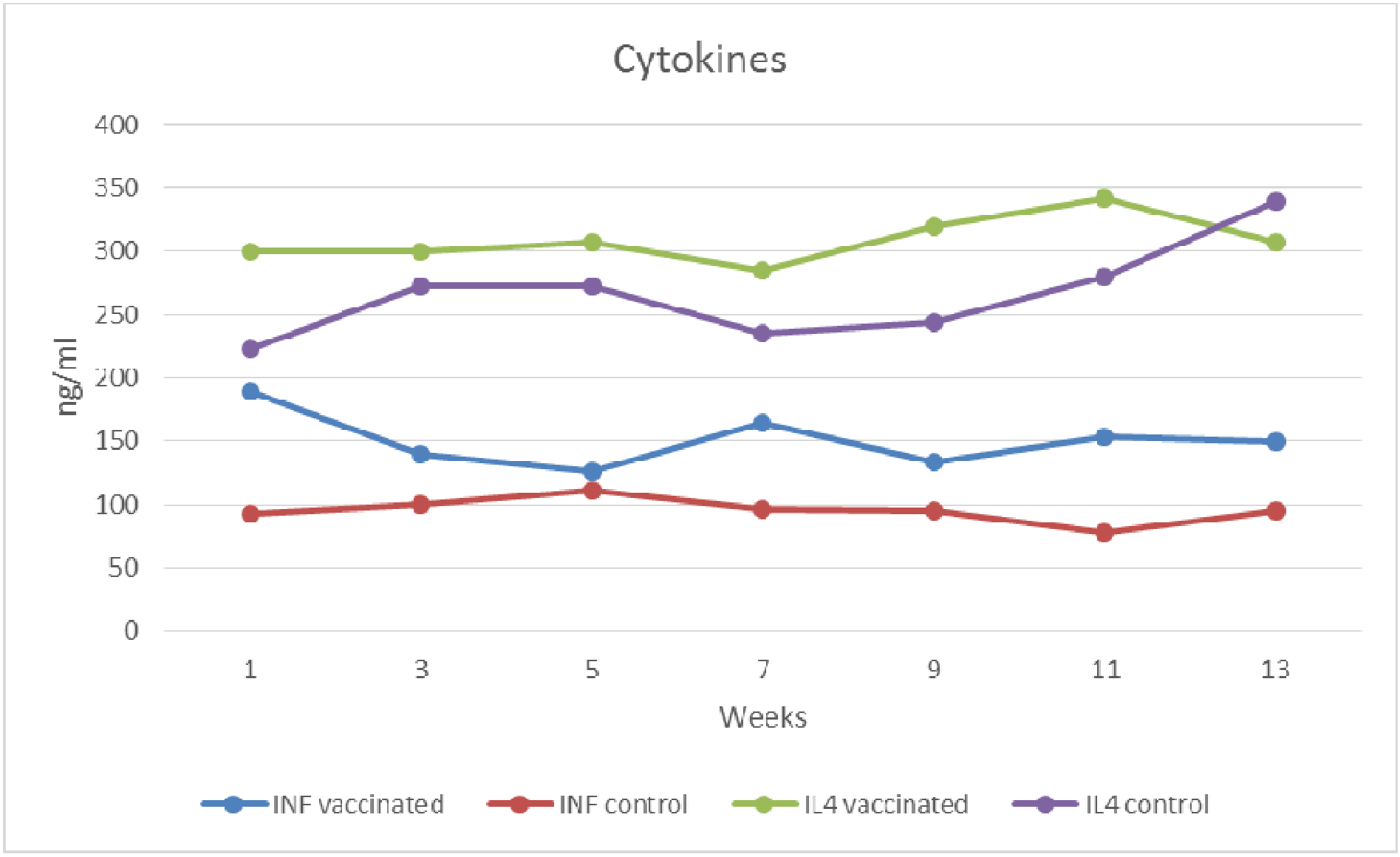
Rabbit INFγ


INF levels
200
150
100
50
0
ng/ml
1
3
5
7
9
11
13
Weeks
INF vaccinated
INF control

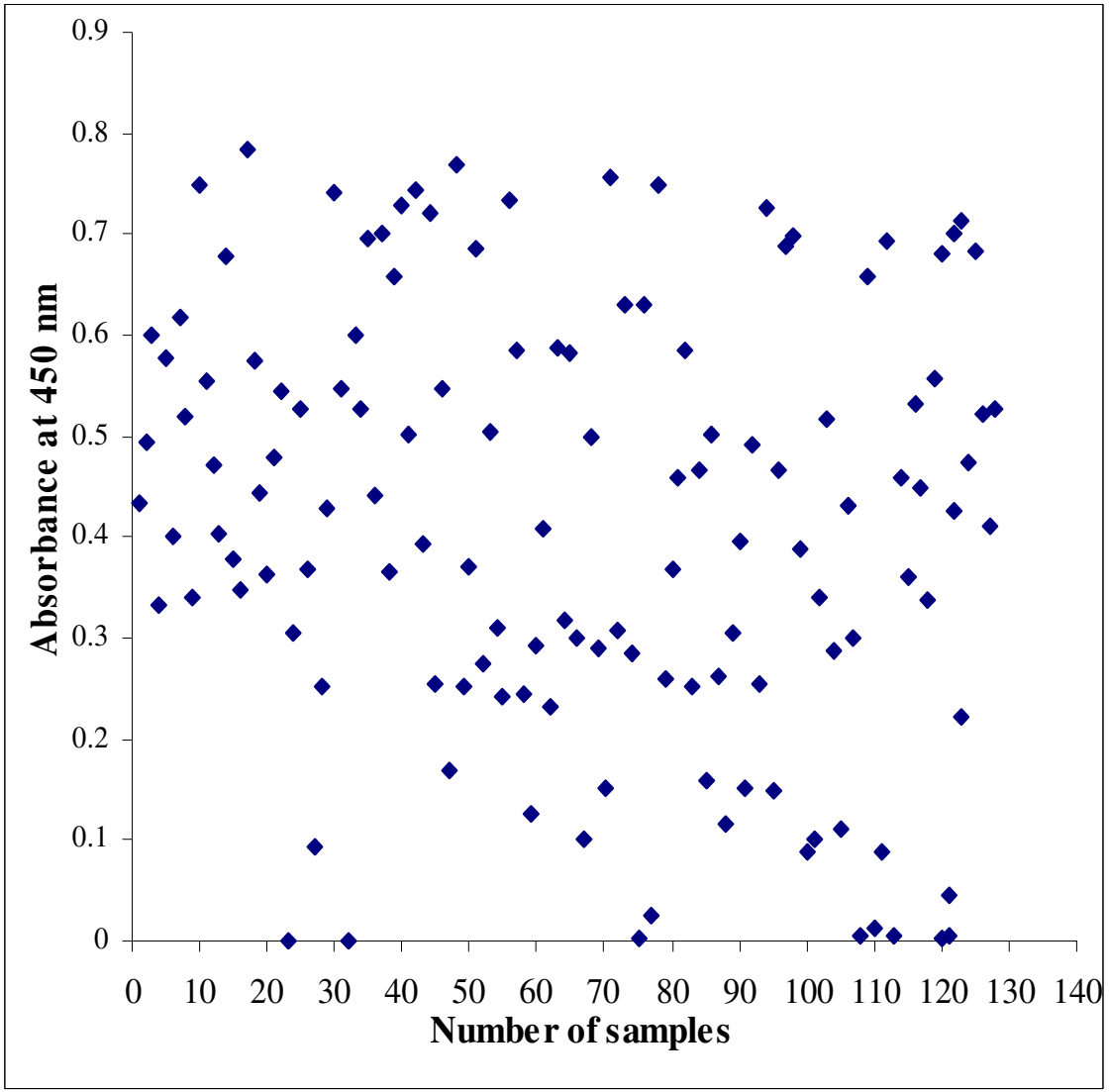
Rabbit IL-4


IL4 level
350
300
250
200
150
100
50
0
ng/ml
1
3
5
7
9
11
13
Weeks
IL4 vaccinated
IL4 control

Levels of both INFγ& IL-4



Fraction diagnostic value of bovine fasciolosis



Concluding Remarks

ES fraction of molecular weight 27.5 and 23KDa Induced protective effect against fasciolosis

The protective effect was proved

Parasitologically; worm burden reduction 85% and reduction in worm length

Immunologically (cellular and Humoral)

Recommendations

Evaluation of different vaccination protocols

Evaluation of other adjuvants

Evaluation of protective effect in other hosts

Thank you

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