
المركز القومي للبحوث
NATIONAL RESEARCH CENTRE

# National Research Centre

# Significance

## Can you eat raw pork?

## Can trichinosis kill you?

**Trichinellosis is a widespread and serious parasitic zoonosis.**

**This disease is acquired by eating inadequately cooked or raw pork or other animal meat containing muscle larvae of the Trichinella parasite.**

TRICHINELLA SPIRALIS

**Human trichinellosis occurs in more than 55 countriesaround the world, and trichinellosis is considered to be a re-emerging disease in some parts of the world due to changes in diet and cooking practices and increasing meat consumption.**

# Risk of eating raw pig meat

**Parasite 2016**, **23**, *27Short Note*

## Inadequate labeling of pork sausages prepared in Corsica causing a trichinellosis outbreak in France


**By**

**Caroline Ruetsch, Pascal Delaunay, Alexis Armengaud, Françoise Peloux-Petiot, Jean Dupouy-Camet,Isabelle Vallée, Bruno Polack, Pascal Boireau and Pierre Marty**

# A 30 KDa mannosyle glycoprotein in the diagnosis of experimental trichinosis in rats

By

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# Taxonomy

Kingdom: Animalia

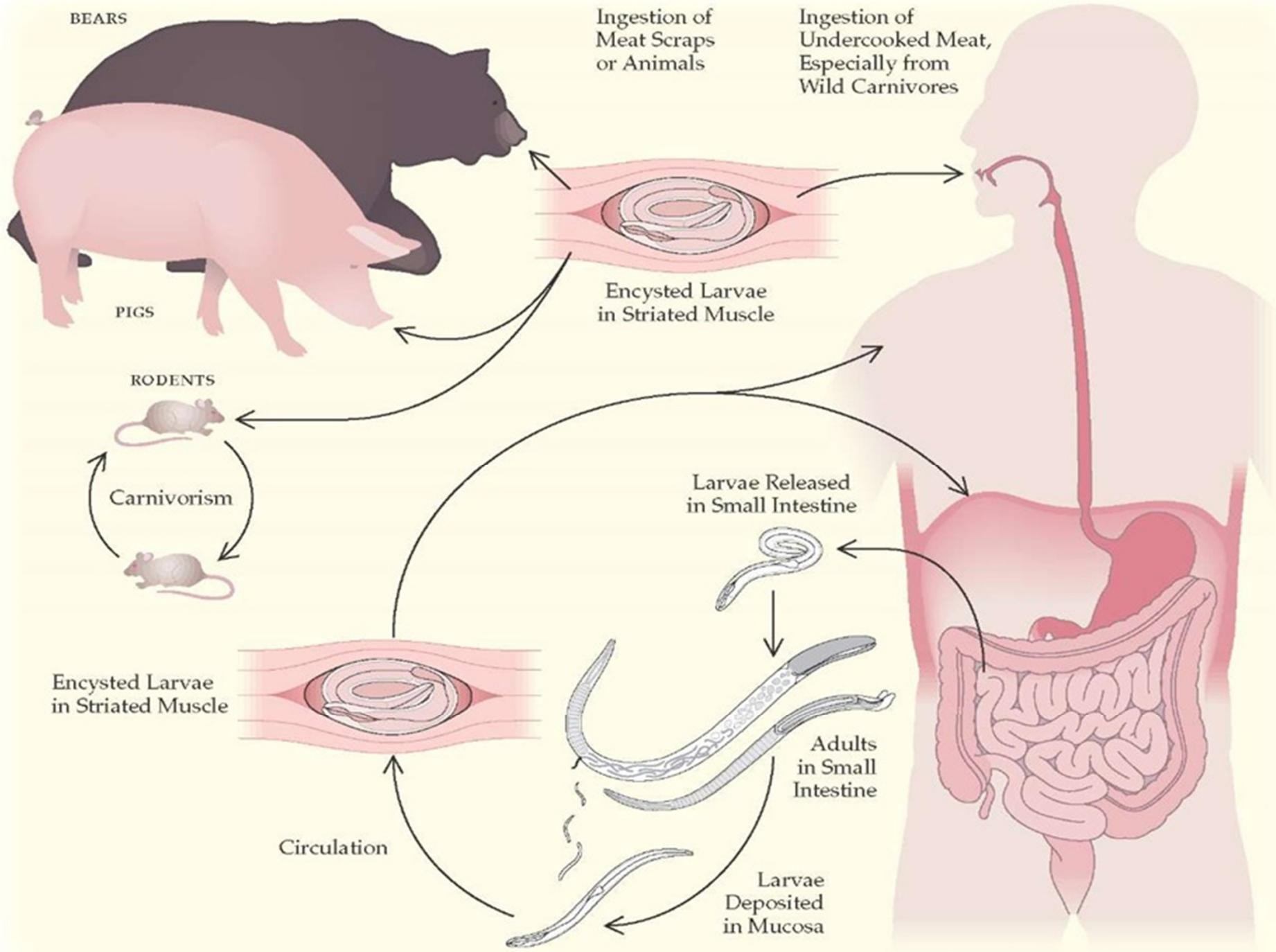
Phylum: Nematoda

Class: Adenophorea

Order: Trichurida

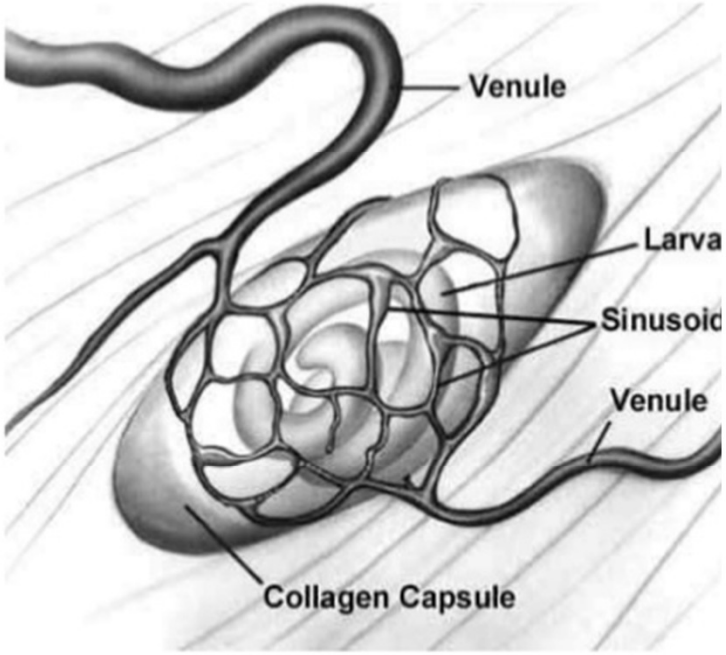
Family: Trichinellidae

Genus: *Trichinella*


BEARS
PIGS
Ingestion of
Meat Scraps
or Animals
Ingestion of
Undercooked Meat,
Especially from
Wild Carnivores
Encysted Larvae
in Striated Muscle
RODENTS
Carnivorism
Larvae Released
in Small Intestine
Encysted Larvae
in Striated Muscle
Adults
in Small
Intestine
Circulation
Larvae
Deposited
in Mucosa

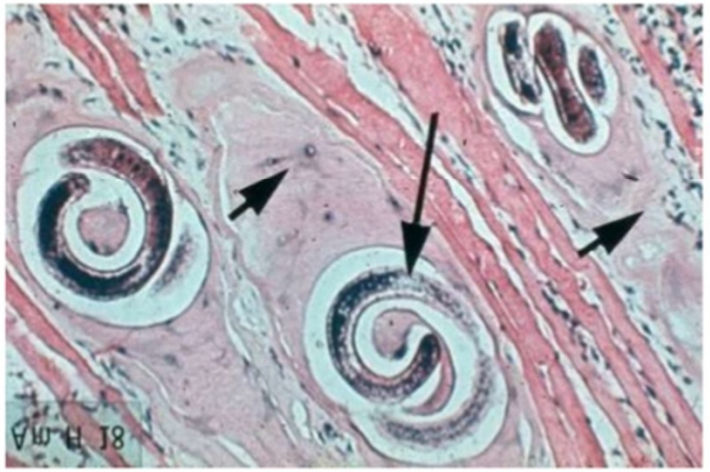
# Trichinella Cyst

## Trichinella spiralis-Trichinella cyst




## Trichinella spiralis-Pathogenesis

- Final stage



# Symptoms

## Enteral phase

**Small intestines symptoms as nausea,heartburn, dyspepsia, and diarrhea from two to seven days after infection.**

**Eosinophilia presents early and increases rapidly.**

# Symptoms

## Parenteral phase

larval migration from the intestines to muscle tissues result in body's inflammatory response; edema , muscle pain, fever, and weakness.

A classic sign of trichinosis is periorbital edema, swelling around the eyes, which may be caused by vasculitis.Splinter hemorrhage in the nails is also a common symptom.

They may very rarely cause enough damage to produce serious neurological deficits (such as ataxia or respiratory paralysis).

Trichinosis can be fatal depending on the severity of the infection; death can occur 4–6 weeks after the infection, and is usually caused by myocarditis, encephalitis, or pneumonia.

# Sins and Symptoms

# Epidemiology

**Traditionally, the epidemiology of *Trichinella* in domestic livestock is limited to pigs.**

**However, the risk of infection through other hosts still there, since sheep, horses,goats,cattle and Ostriches proved to be infected with trichinosis and became source for human infection.**

**This followed the use of proteins of animal origin in breeding herbivorous animals, which is now common practice in many countries.**

# Treatment

**Drug Adult and pediatric dose**

**Albendazole** 400 mg twice a day by mouth for 8 to 14 days

**Mebendazole** 200 to 400 mg three times a day by mouth for 3 days, then 400 to 500 mg three times a day by mouth for 10 days

# Prevention

Properly cooking pork and feeding pig only cooked garbage

Pork inspection in slaughter houses using trichinoscope

# Diagnosis

Early and accurate diagnosis of this very serious disease is essential for:

decrease the troubles associated with this infection,

increase the chance of successful therapy and

decrease its prevalence.

# Diagnosis

Attention has been focused on identifying the parasite molecules.

lectin-blot analyses, using lectins with different carbohydrate specificities, have revealed the presence of highly glycosylated proteins on the surface of *T. spiralis* larvae and in the parasite's excretory secretory products.

# Mannon Binding protein

Among these glycoproteins there are some that possess high mannose and branched structures, mostly three- and tetra-antennary N-glycans.

These oligosacharide structures of parasite glycoproteins are very important in provoking host defense mechanisms, both innate and adaptive, against infectious agents.

# Objective

Isolate and identify new diagnostic glycoprotein antigen of Trichinella spiralis larval antigen

# Materials and Methods

## Parasite

Larvae of *T. spiralis* were obtained from infected pig meat proved to be heavily infected with *T. spiralis*, examined by trichinoscope in Cairo abattoir at Basateen.

The infected meat was minced and digested by conventional method of artificial digestion with Pepsin-HCl according to Azab *et al.* (1999)

## Antigen preparation

T. spiralis larvae were homogenized, sonicated and suspended in Tris-EDTA buffer, containing 40 mM Tris, 1 mM EDTA, 0.25 M sucrose, and protease inhibitors (170 µg/ml "PMSF").

# Materials and Methods

## Experimental infection

Thirty six Laboratory bred rats of 140-160 gm were used.

18 rats were experimentally infected with 250 *T. spiralis* larvae per rat and the other were kept as control negative.

The infection was orally and performed by syringe and a plastic tube.

Blood was collected weekly from the rats starting one week post infection until six weeks to get serum samples.

Control negative sera were collected from control negative animals.

# Materials and Methods

## Rabbit hyperimmune serum

About 40 µg/Kg of *T. Spiralis* antigen was mixed with Freund's complete adjuvant and injected subcutaneously into each of 5 rabbits

A booster dose of antigen 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

## Indirect Heamagglutination Assay (IHA)

## Indirect Heamagglutination IhibitionAssay (IHIA)

# Materials and Methods

## Antigen purification

Lectin affinity column was adopted for glycoprotein

Bound fraction was eluted with 0.2 M mannose.

# Materials and Methods

## Enzyme Linked Immunosorbant Assay (ELISA)

ELISA was adopted for time course analysis of antibodies in experimentally infected rats using *T.spiralis* crude and isolated fraction of larval extract.

The optimal antigen concentration, antibody and conjugate dilutions were determined after preliminary checker- board titrations.

# Materials and Methods

## Antigens characterization

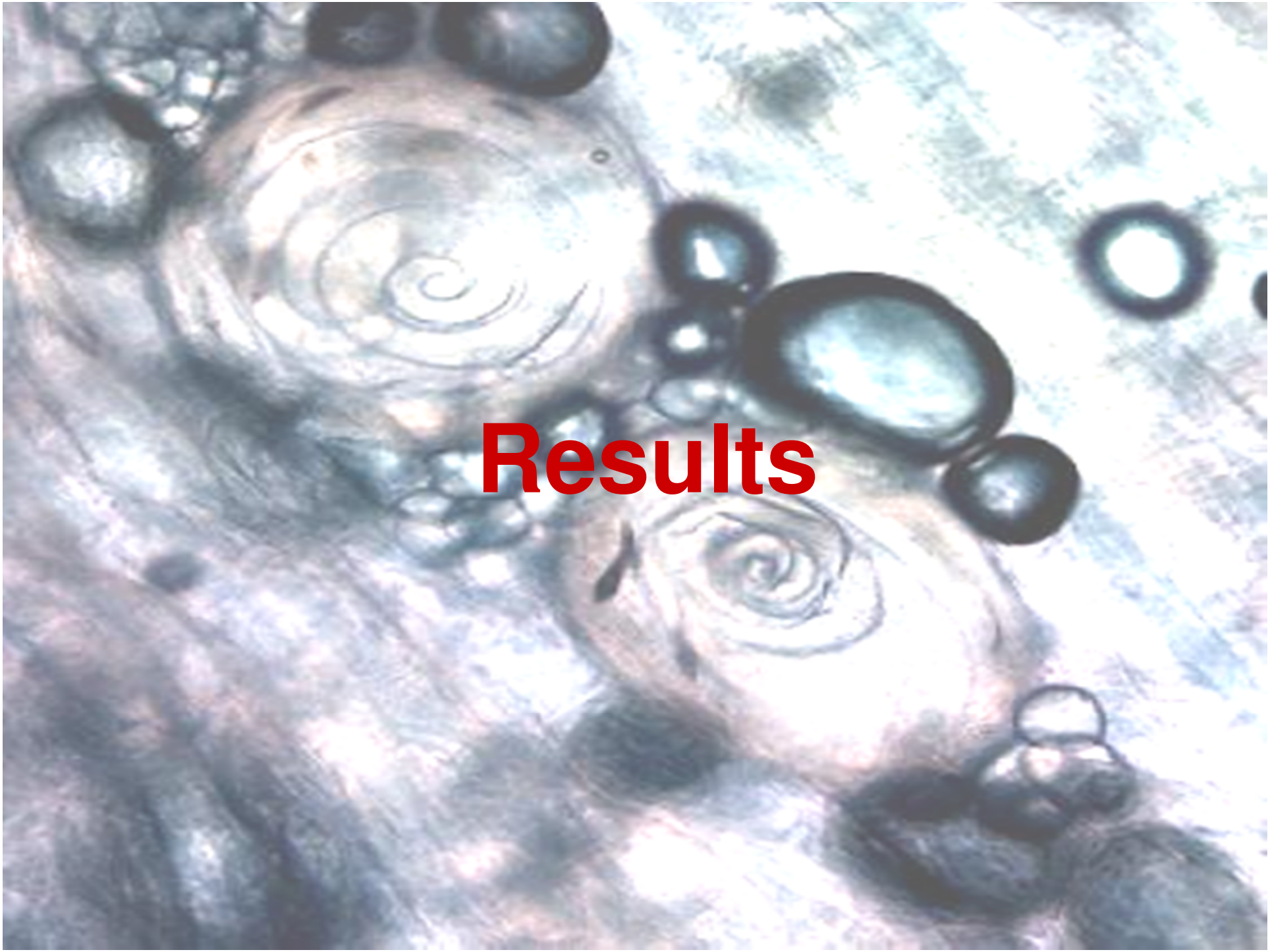
### 1. SDS- PAGE

10% slab SDS-PAGE according to the method of Laemmli (1970)

Gels were stained with silver stain

### 2. Immunoblotting

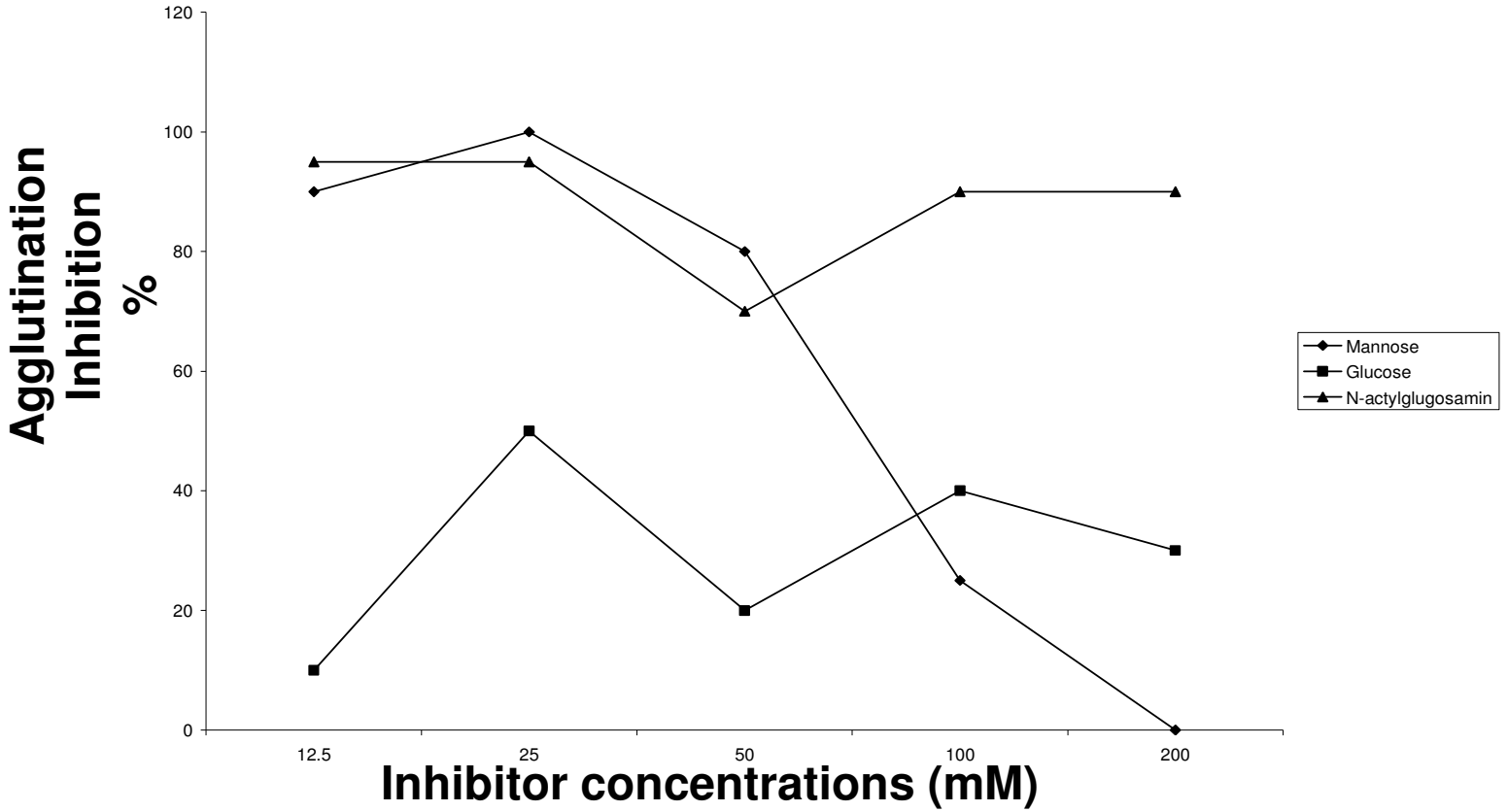
Immunoblot assay was utilized to identify the immunoreactive components recognized in the crude and purified antigens.


Results

# Results
# Indirect Haemagglutination Inhibition

**The most potent monosaccharide associated with *Trichinella* larvae were mannose (100% inhibition at 25 mM), N-acetylglucosamin (95% inhibition at 12.5 mM) and glucose (50% inhibition at 25 mM).**

# Sugars associated with *T.spiralis* larval glycoproteins

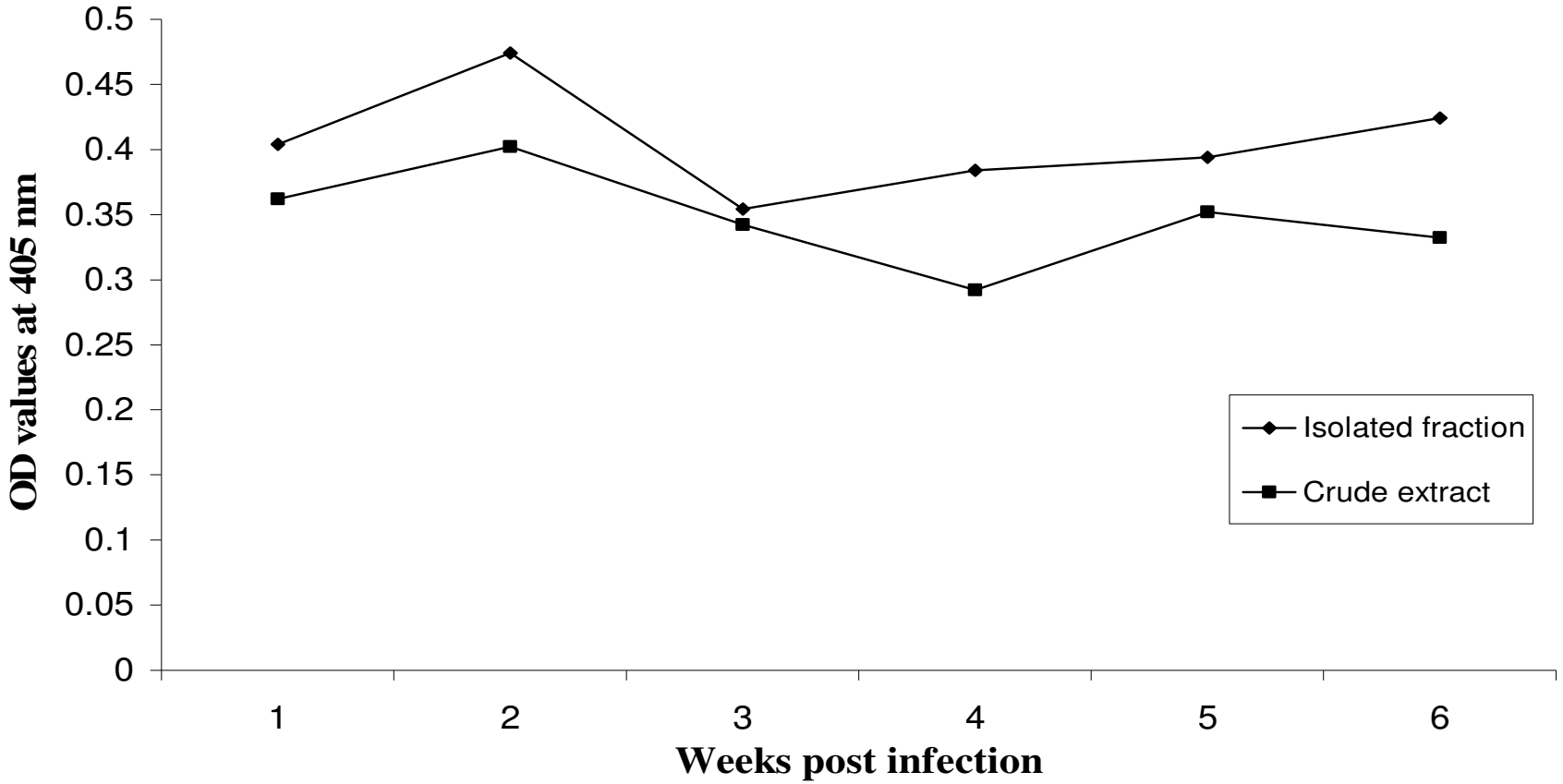


# Purification

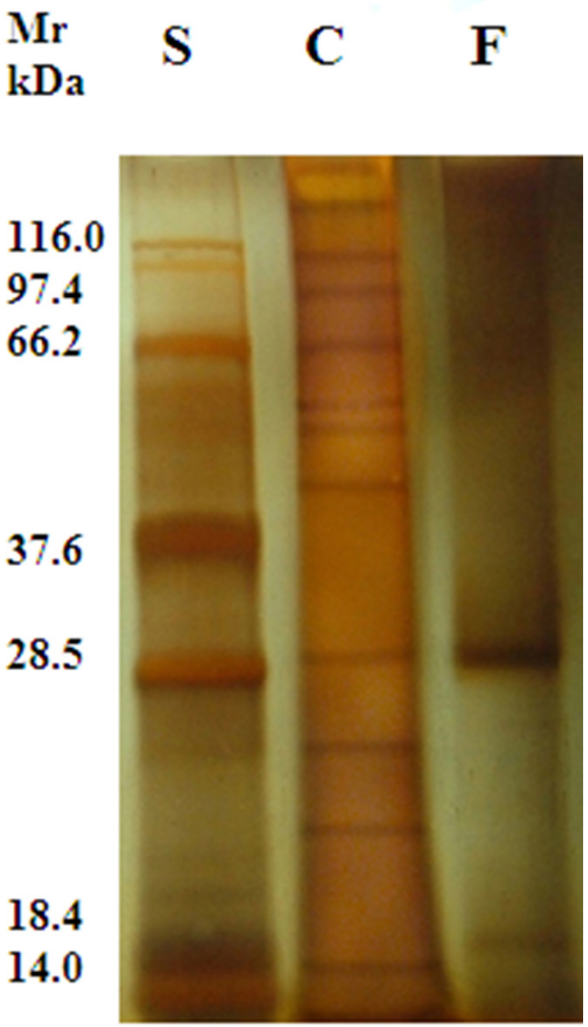
Based on results of **Indirect Heamagglutination Ihibition Assay,**

**Mannose glycoprotein containing component(s) of the crude extract of *T. spiralis* larvae was isolated using concanavalin A-Sepharose 4B (ConA-Sepharose 4B) and the fraction was eluted with 0.2mM mannose**

# ELISA


0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD values at 405 nm
1
2
3
4
5
6
Weeks post infection
Isolated fraction
Crude extract

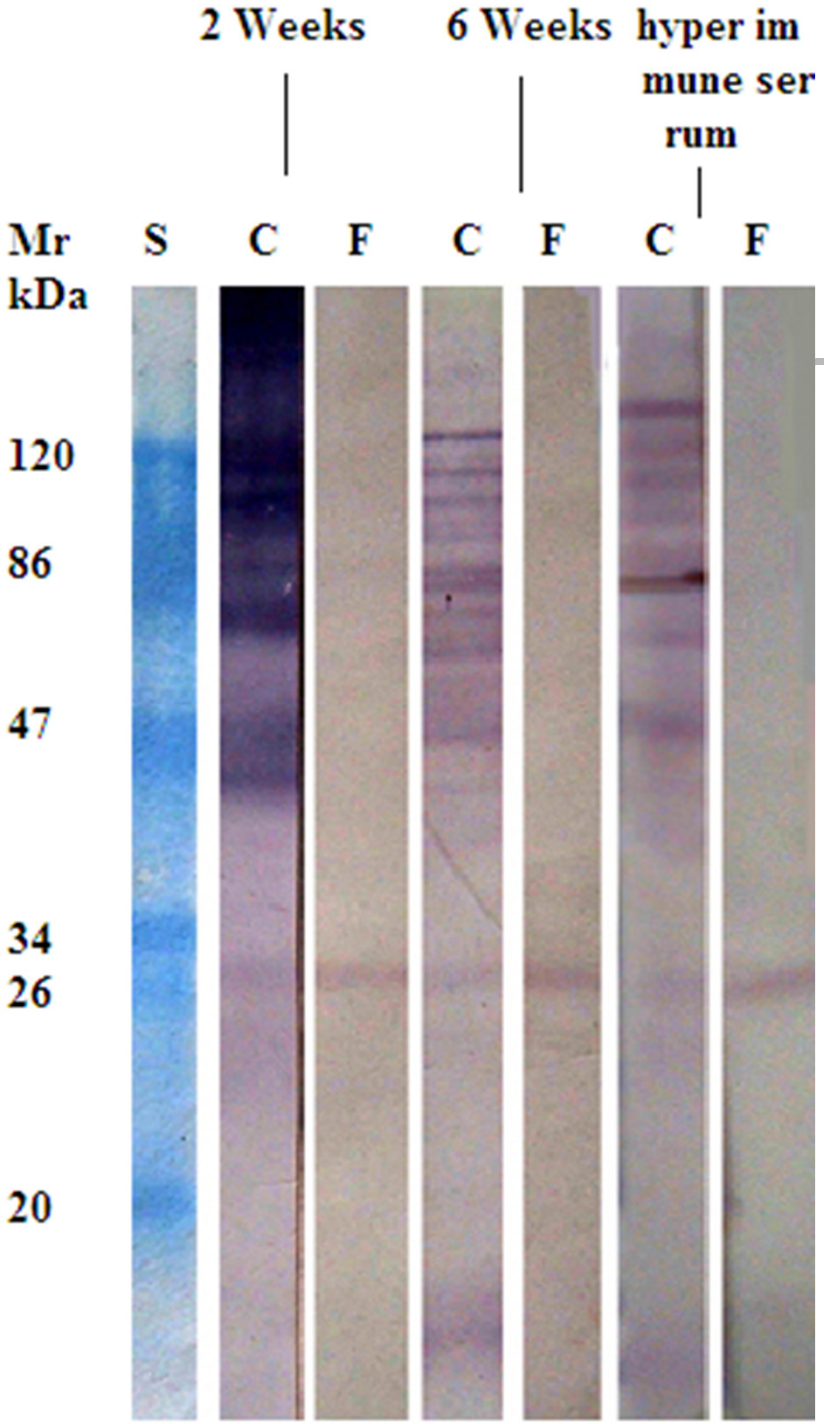
# Electrophoretic profile




4 bands were only revealed in manosyle fraction (Lane F); 65kDa,54kDa,30kDa and 16kDa

Complex profile of Crude extract (Lane C) ranged from 16kDa 132kDa

Lane S for Molecular weight Marker




# Immunoblot

**3o kDa was identified in both crude and fraction using experimentally infected serum; 2weeks and 6weeks PI and hyperimmune serum**

# Concluding Remarks

- The carbohydrate structures that are highly existed in *T.spiralis* encysted larvae were Mannose, N-acetyle glucosamine and Glucose
- Either early in one week P.I. or in the late stage, six weeks P.I., the isolated mannosyl fraction proved higher potency in the diagnosis of experimental trichinosis in rat than crude extract by indirect ELISA.

# Concluding Remarks

a 3o KDa mannosyle glycoprotein(s)was the only identified band in the fraction by different serum samples.

It proved potentials in early as well as late diagnosis of experimental trichinosis in rats

# Recommendations

- The role of the 30 KDa glycoprotein in the immunobiology of *Trichinella* infection, remains to be resolved.
- Further purification of mannosyl fraction introduced in the current study is needed for higher diagnostic potency.

# Recommendations

**Additional studies are also recommended to evaluate the immunodiagnostic role of 30 KDa of *T.spiralis* larvae on a wide range of different hosts; pig, horses, human and food animal.**

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