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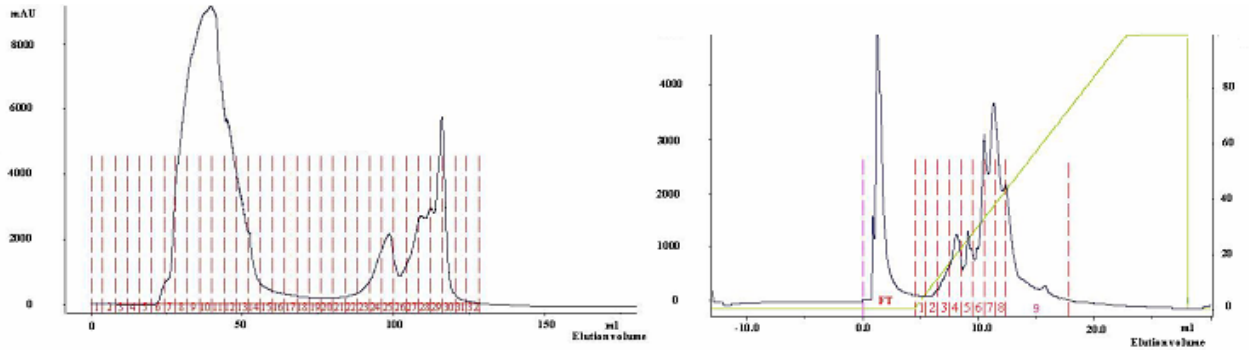
ISOLATION OF A SPECIES-SPECIFIC ANTIGEN FROM *TAENIUM SOLIUM* CYST FLUID AND EVALUATION OF ITS SENSITIVITY AND SPECIFICITY IN ELISA FOR DIAGNOSIS OF PORCINE CYSTICERCOSIS

Ir Assana Emmanuel



A thesis presented for the degree of Master of Science (M.Sc) in Tropical Animal Health

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Dedicated to my family

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Abbreviations

Ab : Antibody
Ag : Antigen
AUC: Area Under Curve
Ab-ELISA : Enzyme-linked-immunosorbent assay for antibody detection
Ag-ELISA: Enzyme-linked-immunosorbent assay for circulating antigen detection
CF: crude cyst fluid
EDTA : Ethyldiamine tetraacetic acid
GP: Glycoproteins
HPLC: High Performance Liquid Chromatography
HR: High Resolution
kDa: Kilodalton
ROC: Receiver-Operating Characteristic
SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis
Se: Sensitivity
Sp: Specificity
TG-ROC: Two graphs-Receiver-Operating Characteristic
TMB: Tetramethyl Benzidine

Summary

Species-specific diagnosis of *Taenia solium* infections in pigs has been hampered so far due to the cross-reactivity with *Taenia hydatigena*, a cestode that also infects pigs. The objective of this study was the purification of a *T. solium*–specific antigen fraction that can be used for immunodiagnosis of porcine cysticercosis. A fraction with a major band of 14 kDa was obtained from crude cyst fluid (CF) of *T. solium* cysticerci by 2-step chromatography. A first fraction isolated by gel filtration (Sephacryl S300 HR) was purified for homogeneity by anion exchange column (Mono Q® HR) with High Performance Liquid Chromatography (HPLC). Evaluation of the analytic sensitivity of this fraction (F3) was carried out in an antibody ELISA (Ab-ELISA-F3) using serum samples from pigs experimentally infected with different doses of *T. solium* eggs. The analytic specificity (cross-reactivity) of F3 was evaluated with serum samples from pigs that were naturally or experimentally infected with *T. hydatigena*, *Taenia s. asiatica, Fasciola hepatica*, *Trichinella spiralis*, *Metastrongylus apri*, *Trypanosoma congolense* and *Sarcoptes scabiei,* and with serum samples of rabbits hyper-immunised with metacestode cyst fluid of *T. hydatigena and T. solium.*

Antibody titres of lightly and heavily infected pigs differed in their kinetics. However, the increase in F3-specific antibodies could not be related to the infection level. Analysis of the specificity of the F3 showed that serum samples of pigs infected with other parasites did not recognise this antigen. In addition, whereas cross-reaction with *T. hydatigena* occurred in ELISA using crude CF as antigen, the F3 antigen fraction was not recognized by rabbit hyper-immune serum samples to *T. hydatigena*.

Evaluation of the diagnostic sensitivity and specificity of the Ab-ELISA-F3 was done by a non-parametric Receiver Operating Characteristic (ROC) analysis using 66 serum samples from Zambian village pigs. The total number of cysticerci of these pigs was determined by dissection (28 pigs harboured *T. solium* cysticerci and 38 were negative at dissection). In addition, 58 serum samples from Cameroonian pigs (28 pigs from cysticercosis-free farms and 30 pigs with cysticerci at tongue inspection) were used in a separate ROC analysis. The results from the non-parametric ROC analysis yielded a low diagnostic sensitivity and specificity (both ≤ 50) with the sera from the Zambian pigs while a relatively high sensitivity and specificity was obtained with the sera from Cameroonian pigs. The main factor contributing to a low diagnostic specificity based on the Zambian serum samples seemed to be the false-positive reactions that were likely caused by the occurrence of transient antibodies in the non-infected animals. The data obtained with the non-parametric ROC method for the Zambian serum samples were confirmed by a new Bayesian ROC approach. This method was developed based on a multinomial distribution and all possible interactions between diagnostic tests. These tests were the Ag-ELISA, Ab-ELISA-CF and Ab-ELISA-F3 for the Zambian pigs and Tongue inspection and Ab-ELISA-F3 for the Cameroonian pigs.

In conclusion, we isolated a fraction with a major band of 14 kDa that showed to be *T. solium* - specific in ELISA. Our results suggest that the F3-antigen is a putative candidate for diagnosis of porcine *T. solium* cysticercosis in the areas where *T. solium*, *T. hydatigena* and *T. s. asiatica* are present. However, in order to compensate for the lower sensitivity and specificity, we advice the combination of the Ab-ELISA-F3 with other serological tests in epidemiological studies. Alternatively, this purified fraction can be used for the production of monoclonal antibodies for the development of a sandwich ELISA that could be used for the species-specific detection of circulating antigens in pig cysticercosis.

Keywords: *Taenia solium*, cyst fluid, purification, ELISA, ROC, Evaluation, Sensitivity, Specificity.

1 GENERAL INTRODUCTION

Cysticercosis is a zoonotic disease caused by *Taenia solium* metacestodes in humans and pigs. This parasite belongs to the family of taeniidae, tapeworms that affect vast numbers of humans and animals (Gause *et al*., 2003). *T. solium* has a two-host life cycle involving an intermediate and a final host. Sexual reproduction occurs in the hermaphroditic adult tapeworm that establishes in the small intestine of humans, who are the only definitive hosts. Mature proglottids containing infective eggs are released with the human faeces. Eggs contain a larval stage known as oncosphere. When eggs are ingested by a pig or by a human, they hatch and oncospheres penetrate the intestinal mucosa and migrate via the circulatory system to a suitable tissue location, particularly striated muscles and the brain where they develop into a mature larva (metacestode) called *Cysticercus cellulosae* (Euzeby, 1998). Further transmission of the parasite occurs when raw or undercooked meat from infected pigs is eaten by men.

Cysticercosis is considered as a major cause of neurological pathology in humans (Tsang & Wilson, 1995; Bern *et al*., 1999; Geerts *et al*., 2002) and a source of enormous economic losses to pig breeders (Zoli *et al*., 2003) in developing countries. Except for Muslim regions, there are many countries where pigs are raised traditionally with environmental conditions that facilitate transmission of *T. solium* cysticercosis. Efforts were made around the world to control this disease, but few countries have been able to eradicate or reduce the infection level. This situation is related to the fact that general improvement of the economic situation of the countries together with improvements in public health and sanitation, provision of latrines and industrialisation of pig-raising are needed to decrease transmission of *T. solium* (Lightowlers *et al*., 2000).

The development of improved diagnostic techniques has contributed to our knowledge on the importance of cysticercosis both in pigs and humans. Accurate diagnosis of human cysticercosis can be performed with magnetic resonance imaging (MRI) and computed tomography (CT), that are also useful to classify neurocysticercosis (NCC) in active, inactive or mixed NCC (Sotelo *et al*., 1985, Carpio *et al*., 1998). However, these techniques are expensive and inaccessible for populations from areas at risk. Many efforts have been made to improve the diagnostic capacities of immunodiagnostic tests (Dorny *et al*., 2003; Ito & Craig, 2003), such as the enzyme-linked-immunosorbent assay (ELISA) (Engvall & Perlman 1971). Immunodiagnosis is more accessible and less expensive than the neuroimaging techniques.

Because pigs are the main intermediate hosts in the *T. solium* life cycle, control measures of cysticercosis require a good diagnostic test to identify the animals which have had contact with *T. solium* eggs and/or which harbour the cysticerci. Common procedures for diagnosis of swine cysticercosis are tongue and carcass examination. These techniques show a high specificity, however, their sensitivity is low (Gonzales *et al*., 1990). The serological diagnosis of porcine cysticercosis includes antibody and antigen detection. Some tests for the detection of antibodies using crude antigen preparation showed a high sensitivity (Biagi & Tay, 1958, Herbert & Oberg, 1975; Pathak *et al*. 1984 and Pinto *et al*., 2000). However, cross-reactions between crude antigens of *T. solium* and other helminths are a major problem (Kumar & Gaur, 1987; Cheng & Ko, 1991). Using heterologous antigens from *Taenia crassiceps,* which can easily be produced on mice, other investigators reported a high sensitivity and specificity in ELISA's for the detection of antibodies (Biondi *et al*., 1996; Pinto et *al*., 2000). Several studies have also shown that low molecular weight glycoprotein's (10 – 26 kDa) isolated from cyst fluid of *T. solium* cysticerci provide high specificity and sensitivity for serological diagnosis of cysticercosis (Gottstein *et al*., 1986; Tsang *et al*., 1989, Ito *et al.*, 1998; Ko & Ng, 1998; Yang *et al.*, 1998). Lentil-Lectin affinity chromatography

and iso-electric focusing were the techniques used to isolate these low molecular weight antigens.

For the detection of circulating antigens, Dorny *et al*. (2003) reported that only monoclonal antibody-based tests directed at defined parasite antigens may ensure reproducibility and specificity. A highly sensitive monoclonal antibody-based sandwich ELISA (Ag-ELISA), which was initially developed for *Taenia saginata* cysticercosis (Brandt *et al*., 1992) has been validated and used in porcine cysticercosis (Nguekam, 1998; Assana *et al*., 2001; Pouedet *et al*., 2002, Shey-Njila *et al*., 2003). However, the monoclonal antibody-based sandwich ELISA is genus- and not species-specific, hence, it also detects infections with *Taenia hydatigena* cysticerci in pigs (Dorny *et al*., 2004a), which may lead to false positive diagnosis of *T. solium* cysticercosis.

The native antigens of cysticerci purified by the different techniques as listed above are not produced in sufficient quantity for general use around the world and, available diagnostic kits based on these antigens are expensive. For this reason, we initiated to purify cyst fluid of *T. solium* cysticerci by high performance liquid chromatography (HPLC) to obtain native antigens for local use in the diagnosis of *T solium* cysticercosis in Cameroon. The main objectives of this work were:

- Isolation of a species-specific low molecular weight antigen fraction (between 10-26 kDa) from cyst fluid of *T. solium* cysticerci;
- Evaluation of analytic and diagnostic sensitivity and specificity of the purified antigens in an ELISA for the detection of circulating antibodies against *T. solium* cysticerci (Ab-ELISA).

2 ISOLATION OF A LOW MOLECULAR WEIGHT PROTEIN FRACTION FROM CYST FLUID OF *T. SOLIUM* CYSTICERCI

2.1 Introduction

2.1.1 Main methods in proteins purification

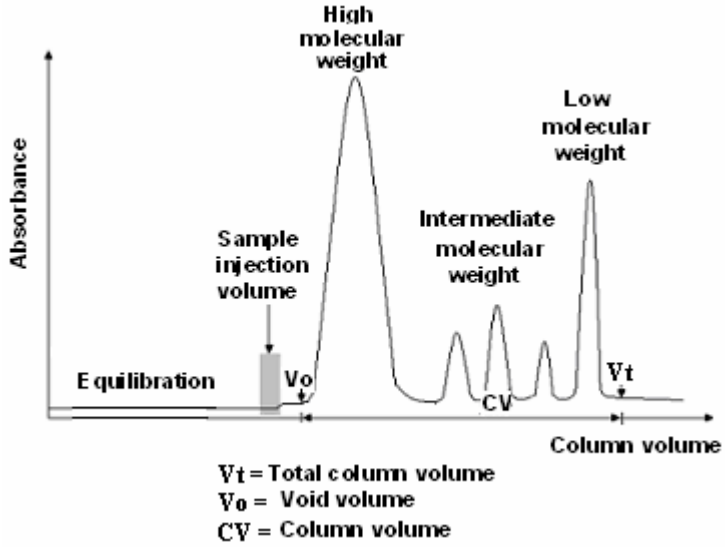
To understand the logic of protein purification, it is important to know that there are a broad range of approaches that can be used to separate proteins on the basis of their physical or biochemical properties (Coligan *et al*., 2001). It is beyond the scope of this review to describe protein fractionation. We present here 3 main approaches for separation and purification of proteins: ion exchange, hydrophobic interaction and gel filtration. To purify a protein one must begin with starting material and fractionate it using any one of a large number of approaches.

-Ion exchange chromatography: Ion exchange chromatography (IEC) is a mode of chromatography in which separation is based mainly on the difference in ion affinity of sample components.

-**Hydrophobic chromatography**: This kind of chromatography is based on interactions between solvent-accessible non-polar groups on the surface of biomolecules and the hydrophobic ligands covalently attached to column matrix.

-Gel filtration: Gel filtration chromatography is a chromatography technique that allows separation of proteins on the basis of size. The sample is applied on a column consisting of a porous matrix chosen for its inertness and chemical and physical stability. Figure I shows the theoretical elution profile of gel filtration chromatography with a high resolution fractionation (Gel filtration handbook, Amersham Biosciences). The small molecules enter the matrix, but large ones cannot. The result is that large molecules flow rapidly through the column and emerge first. Proteins of intermediate size are partially included- meaning they can fit inside some but not all of the pores in matrix. These proteins will then elute between the large and small proteins.

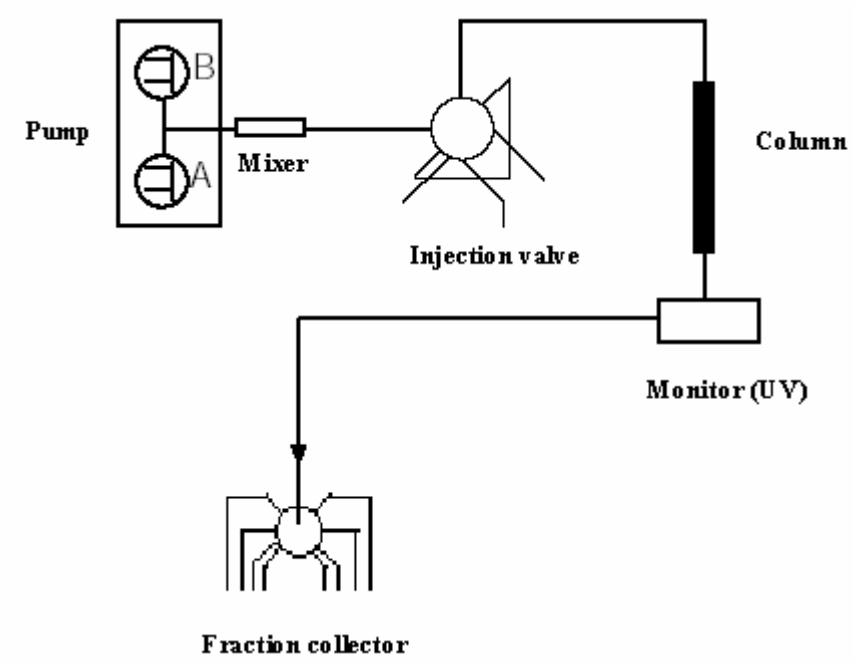
Figure I: Theoretical gel filtration chromatogram with high resolution fractionation (UV absorbance)



2.1.2 High performance liquid chromatography

High performance liquid chromatography or HPLC is a term which refers exclusively to reversed-phase chromatography or includes all sorts of chromatography, provided that the equipment is fully automated and high performance adsorbents are used (Coligan *et al*., 2001). Common HPLC is a system in which the components (Figure II) are controlled by software. Amersham Biosciences ÄKTA design systems, a range of liquid chromatography systems, are controlled by UNICORN^{TM} software, which ensures simple communication between system and user (ÄKTA explorer chromatography handbook, Amersham Biosciences).

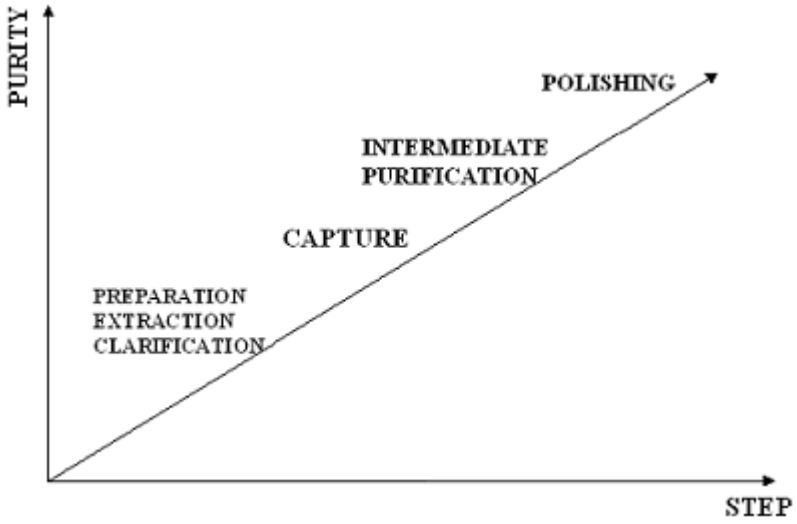
Figure II: Flow configuration of HPLC system



2.1.3 Strategies for protein purification

After preliminary preparation of a crude sample to obtain clean and clear start material, the purification strategy for any protein at any scale of operation can be divided into three sequential stages (Figure III): capture, intermediate purification and polishing (Coligan *et al*., 2001). The capture stage is the initial purification of the target protein from the source material. The goal of the capture stage is to concentrate the target protein while removing as much of the major contaminants as possible. Once the solution containing the target has been clarified and concentrated, the next stage in the purification process is the intermediate purification. Polishing is the final stage in the purification process. The goal of polishing is to remove structural and functional variants of the target protein.

Figure III: Preparation and three phases of purification strategies



2.2 Materials and methods

At the beginning of this work on cyst fluid purification, we had no planned chromatographic strategy to isolate low molecular weight proteins. We tried some methods with different kinds of columns such as ion exchange chromatography, hydrophobic interaction and gel filtration. Some of these methods gave unsatisfying results and are therefore not presented here. In this work we describe the two methods that gave the best results in terms of isolation of a low molecular weight protein fraction from crude cyst fluid (CF).

2.2.1 CF preparation

Fresh cysticerci were removed from naturally infected pigs. The host membrane tissues, which covered the cysticerci were gently removed. After several washings (more than 3 times) in 0.15 M NaCl, the cysticerci were stored at -30 °C until fluid extraction. The CF was aspired with a needle from the intact and thawed cysts, pooled and centrifuged at 18000 x g for 30 min at 4 °C. The supernatant was collected, lyophilized and stored at 4 °C until further processing.

2.2.2 First step of cyst fluid purification: fractionation of CF

- HPLC system

An automated liquid chromatography system (ÄKTA basic 100, Amersham Pharmacia Biotech, Uppsala, Sweden) with a fraction collector (Frac 901, Amersham Pharmacia Biotech) was used to separate proteins from CF. The system was controlled by UNICORN Software (UNICORN V3, Amersham Pharmacia Biotech).

-Fractionation by gel filtration

Forty milligrams (mg) of lyophilized CF was dissolved in 5 ml of Tris buffer (20 mM Tris-Base) and loaded on a gel filtration column (Sephacryl S-300, Amersham Pharmacia Biotech, Uppsala, Sweden) through the injection valve connected to a sample loop of the HPLC system. The system was run at room temperature at a flow rate of 0.5 $ml.min^{-1}$. A Tris-

saline buffer (0.15 M NaCl in 20 mM Tris) was used as elution buffer. A UV monitor connected to the HPLC system was used to detect the eluted (UV-1, 280 nm). The fraction collector collected fractions of 2 ml. The collected fractions of the same peak were pooled, dialyzed and lyophilized. Analysis of the proteins was performed with 12 or 15 % standard polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). The gel was stained with Coomassie Brilliant Blue R250 (SIGMA) or Silver-stained. Molecular weight markers from MBI Fermentas (Germany) and from Amersham Pharmacia Biotech (Amersham Pharmacia Biotech, Uppsala, Sweden) were used as molecular weight reference for Coomassie Brilliant Blue and Silver-staining, respectively. After electrophoresis analysis the peak containing major protein bands of 10-26 kDa was retained to future purification.

-Fractionation on anion exchange column (Mono Q HR)

Ten milligrams (mg) of lyophilized CF were dissolved in 0.5 ml of Tris buffer and applied to a sample injection connected to a sample loop and the anion exchange column (Mono Q HR 5/5, Amersham Pharmacia Biotech, Uppsala, Sweden). Buffer A (Tris buffer) and buffer B (1M NaCl in Tris buffer) were used, respectively as equilibration buffer and elution buffer. The fraction was eluted by gradient concentration of buffer B (0 to 100 % B) in buffer A at a flow rate of 1 $ml.min^{-1}$ at room temperature. The gradient length of 20 ml was programmed using Unicorn software. The eluted protein was detected as described for the gel filtration. The size of collected fractions was 1.75 ml per tube. The uncollected fractions were deflected by the outlet valve. The collected fractions of the same peak were pooled and dialyzed against Tris 20 mM (overnight at 4°C). A protein assay kit (SIGMA diagnostics) determined the protein concentrations of the pooled peaks. The pooled fractions were also analyzed by electrophoresis as described for the gel filtration.

2.2.3 Second step of CF purification

The second step could be considered as a polishing step. The fractions obtained by gel filtration in the first step and showing bands of low molecular weight on SDS-PAGE were dialyzed against Tris buffer and lyophilized. Then the lyophilized fractions were dissolved to the appropriate concentration in Tris buffer and purified by ion exchange chromatography using a Mono Q HR (anion exchange column) or a Mono S HR column (cation exchange column). The low molecular weight fractions obtained by the anion exchange column in the first step were also purified by gel filtration.

In summary, we developed two methods based on the combination of gel filtration and ion exchange chromatography (Figure. IV):

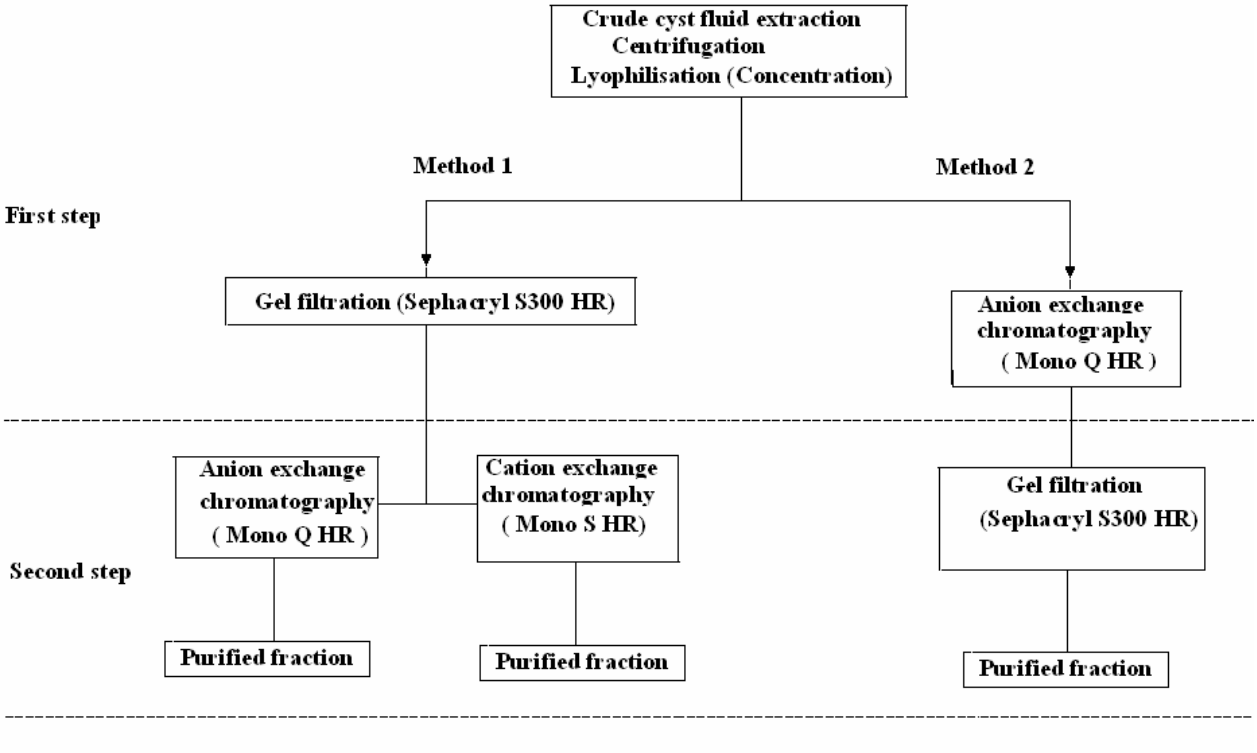
-the first purification consisted of gel filtration, followed by anion or cation exchange chromatography;

-the second purification was initiated by gel filtration and ended by anion exchange chromatography.

2.2.4 Determination of glycoprotein components in F3

Detection of carbohydrates components in the purified fraction was done using the orcinol reaction on thin layer chromatography plates (TLC plates). Briefly, five µl of F3 was applied on a TLC plate (POLYGRAM Sil GUV/254, Macherey Nagel, Germany). An equal volume of glucose was spotted on the plate as positive control. After 2 hours incubation at room temperature, the plate was briefly sprayed with 0.1 % orcinol dissolved in 5 % H_2SO_4. Thereafter the membrane was heated at 100°C for approximately 5-10 minutes. Using this reaction spots containing sugar appear pink/violet on a violet background.

Figure IV: Schematic representation of *T. solium* antigen purification on HPLC



2.3 Results

2.3.1 First step of purification: Fractionation of CF

-Fractionation on gel filtration column

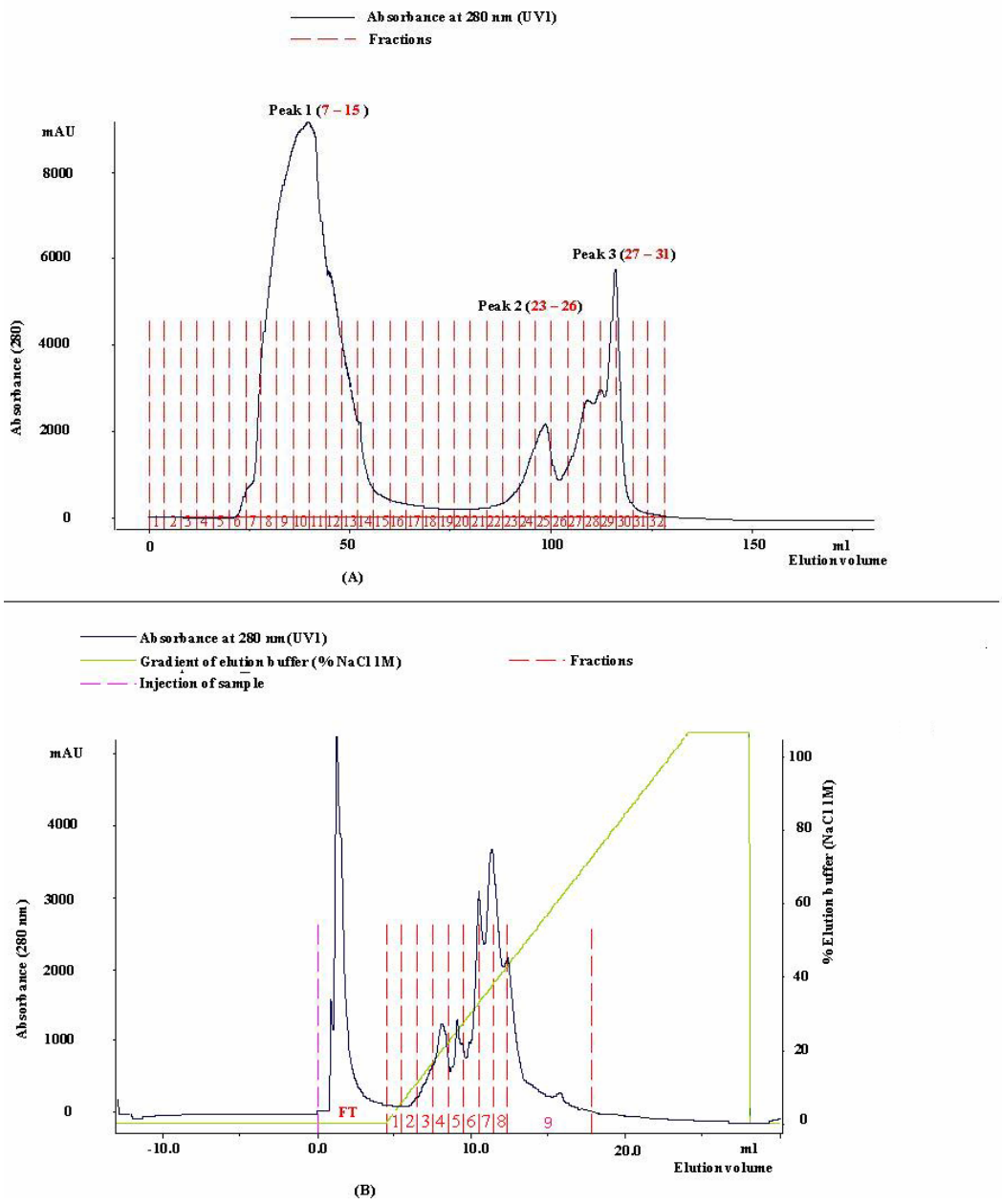
Three major absorbing peaks were resolved by gel filtration (Figure. VA). The eluted fractions, corresponding to each peak, were collected separately (Peak1, Peak2, Peak3). Several purification runs were made and the eluted fractions corresponding to each peak were collected and pooled.

The three peaks obtained during the purification of CF on gel filtration were also analysed by SDS-PAGE. The profiles of the three parts of the first peak (Fraction 8, Fraction 10, and Fraction 13) were basically similar and several protein bands were observed between 10-116 kDa (Figure VIC). The second peak showed a few prominent bands between 14 and 20 kDa (Figure VIB), while the third peak showed a single prominent band at 14 kDa (Figure VIA).

- Fractionation on anion exchange column

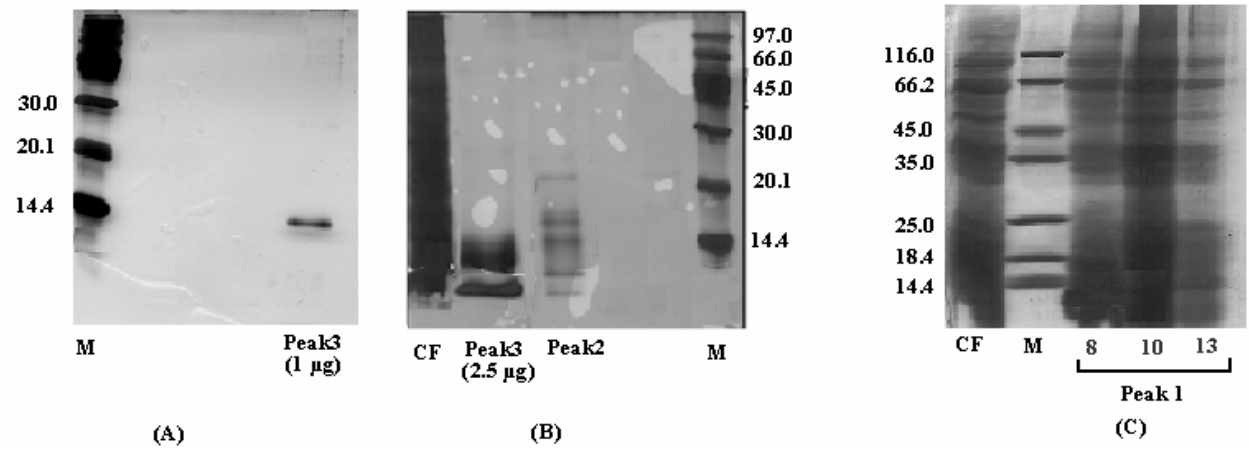
Figure VB shows the profile of CF fractionated by anion exchange chromatography. Nine fractions were obtained with a gradient of buffer B (0-100%) in buffer A. Fractions 5, 6 and 7 contained a protein band of 14 kDa as detected with SDS-PAGE (Figure VII). These fractions were pooled (F1) for further purification by gel filtration.

Figure V: Fractionation of CF by gel filtration and anion exchange chromatography



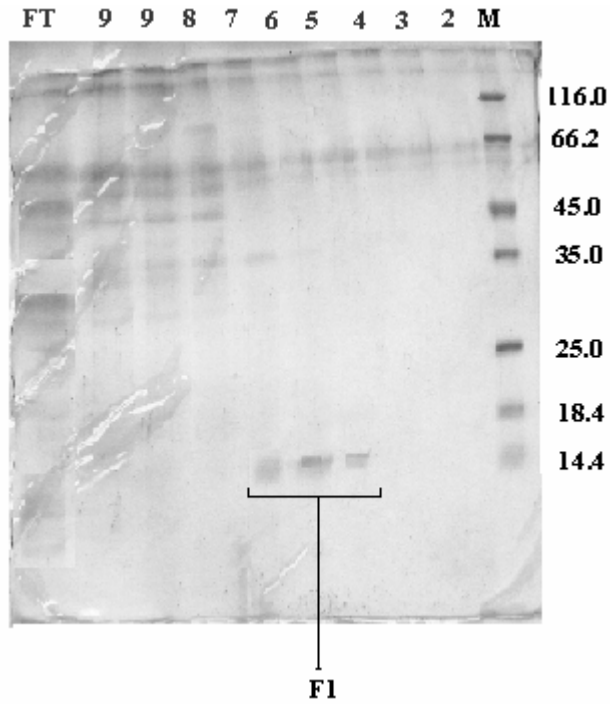
(A): Fractionation of CF on Sephacryl S-300 HR (gel filtration).
(B): Fractionation of CF on Mono Q HR (anion exchange chromatography).
Unbound proteins or flow through (FT) were also collected.
The absorbance value is depicted on the Y-axis and is relative to the protein concentration in the fractions.
The X-axis shows the eluted volume in relation to the number of the fraction.

Figure VI: SDS-PAGE (15 % gel) of major gel filtration peaks and crude cyst fluid (CF)



(A) : Silver-staining of Peak 3; 1 µg of protein was loaded. One major band was observed at the level of 14 kDa. The M lane contained a protein marker.
(B) : Silver-staining of crude CF, Peak 2 and Peak 3 lane contained 1 and 2.5 µg respectively. 5 µl of crude cyst fluid were loaded (CF lane). This SDS-PAGE confirmed that Peak3 did not contain a protein band above 14 kDa.
(C) : Coomassie brilliant blue R250-stained SDS-PAGE of 3 fractions of Peak1: 15 µl of each fraction was loaded on the gel. 10 µg of CF was loaded. Lane M contained protein standard.

Figure VII: SDS-PAGE (10 % running gel) of different fractions obtained by anion exchange chromatography



Fractions in lanes 5, 6 and 7 contain a band at 14 kDa. These 3 fractions were pooled (F1) for further purification by gel filtration .

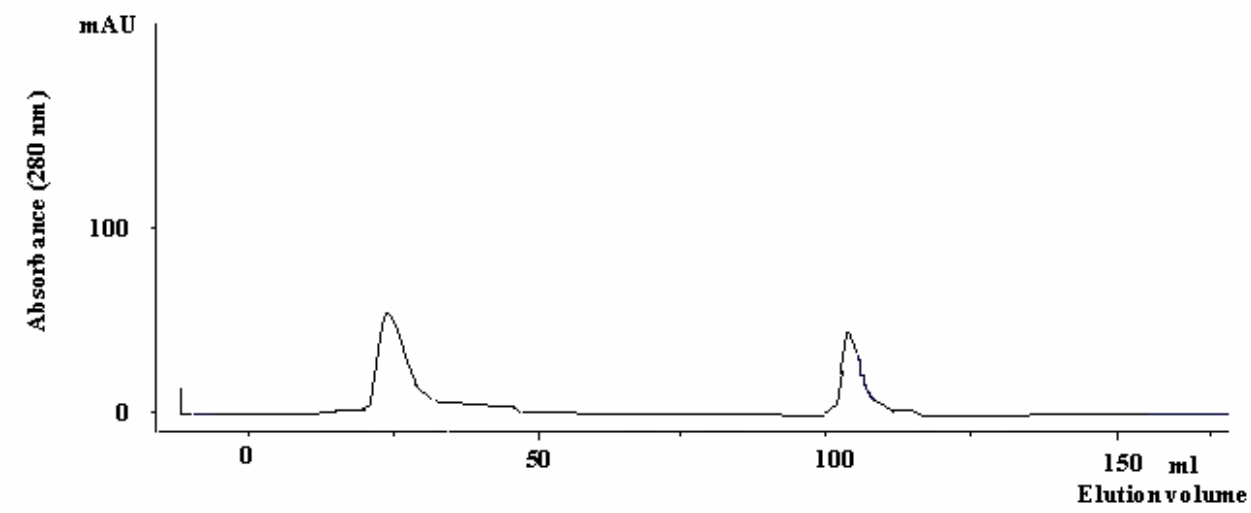
2.3.2 Second step of the purification

The fractions containing low molecular weight protein bands from the first purification, were polished in a second purification step. F1 obtained by anion exchange chromatography in the first step was purified by gel filtration, while Peak2 and Peak3, obtained by gel filtration, were purified by ion exchange chromatography using Mono Q HR column (anion exchange column) or Mono S HR column (cation exchange column).

-Purification of F1 by gel filtration

Figure VIII shows the profile of F1 on gel filtration. The first peak represents proteins with high molecular weight and the second peaks represents low molecular weight proteins (SDS-PAGE not shown). However, the recovered amount of proteins in the fractions was very low: it was estimated that 99 % of the proteins of the initial cyst fluid were lost (by comparing the absorbency of the start material to the absorbency of the final purified fraction).

Figure VIII: Profile of F1 fractionated by gel filtration

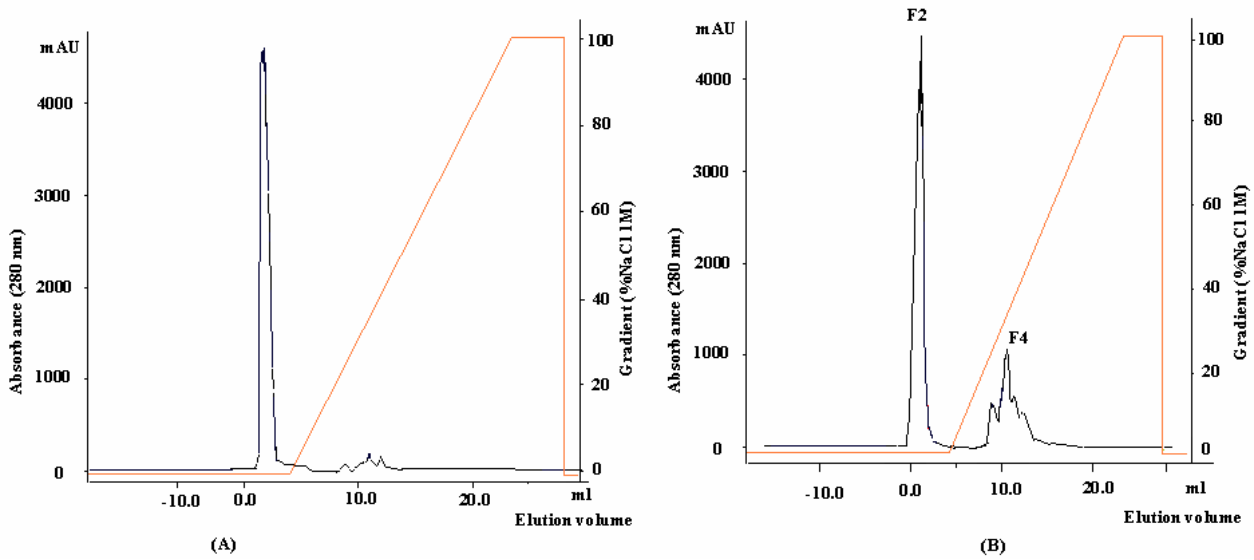


-Purification by ion exchange chromatography and characterisation of Peak 2 and Peak 3

Only a few proteins of Peak 2 were bound on the Mono S column (Figure IXA) whereas anion exchange chromatography (Figure IXB) showed more bound proteins. However, the protein concentration of the bound fraction (F4) as measured by absorbency, was lower than the protein concentration of the unbound fraction (F2). Hence, only the unbound fraction was further analysed by SDS-PAGE (Figure XIB). SDS page revealed that the protein profiles of F2 and Peak 2 were similar, displaying protein bands between 14-20 kDa.

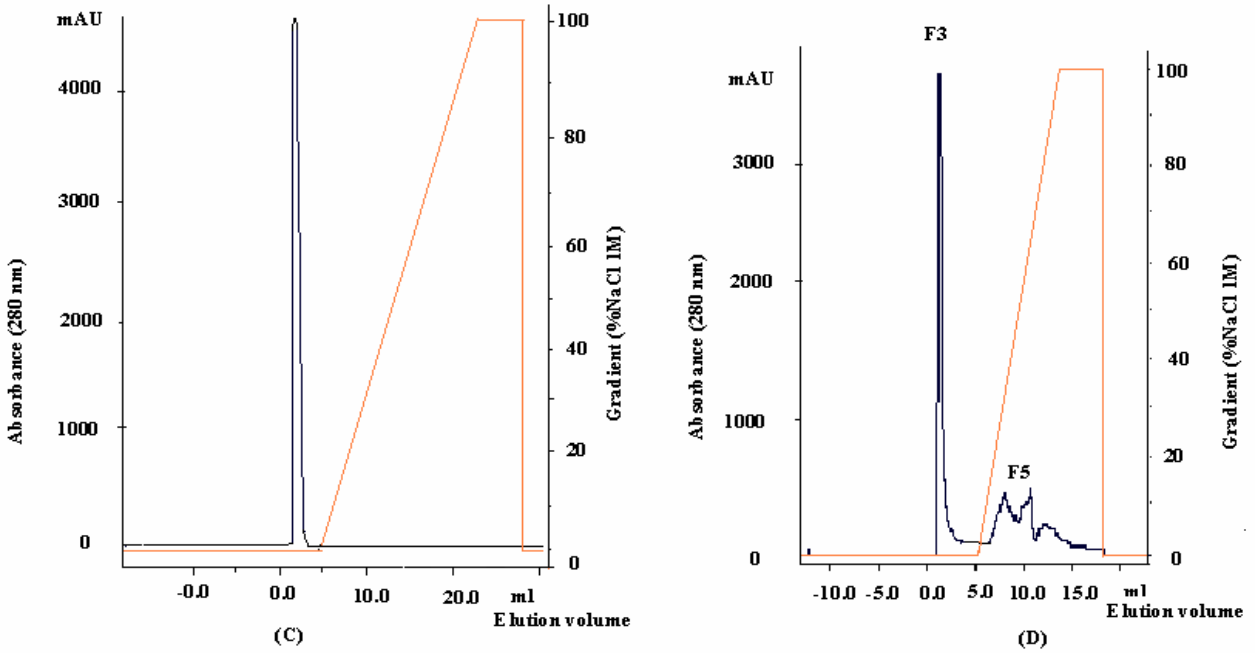
Peak 3 did not bind to the cation exchange column (Figure XC) and showed only a small amount of bound proteins on the anion exchange column (Figure XD). This suggests that Peak 3 contains proteins with homogenous characteristics. Coomassie brilliant Blue dye and Silver-staining following SDS-PAGE of the unbound fraction showed a prominent protein band at 14 kDa (F3). Based on the apparent purity of this protein fraction, F3 was selected as antigen for further evaluation in an Ab-ELISA. The fraction of Peak 3 that bound on the on anion exchange column (F5) was not further analysed with SDS-PAGE.

Figure IX: Elution profiles of the cation and anion exchange purification of Peak 2



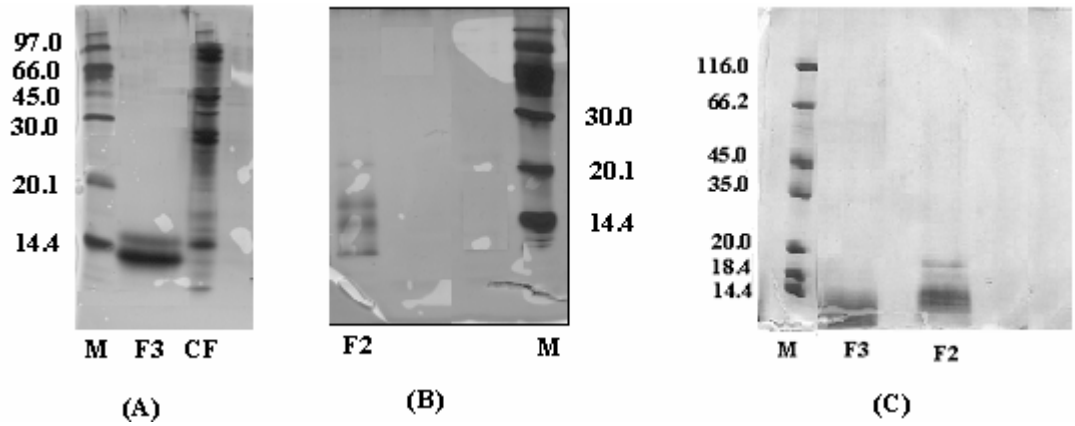
(A) : Elution profile at 280 nm of cation exchange purification of Peak 2 on Mono S (HR).
(B) : Elution profile at 280 nm of anion exchange purification of Peak 2 on Mono Q (HR). The major peak (F2) from Mono Q HR was considered as the purified fraction peak and was the unbound fraction (F2). F4 was the bound fraction.

Figure X: Elution profiles of the cation and anion exchange purification of Peak 3



(C): Elution profile at 280 nm of cation exchange purification (Mono S HR) of Peak 3
(D): Elution profile at 280 nm of the anion exchange purification (Mono Q HR) of Peak 3

Figure XI: SDS profile of F2 and F3

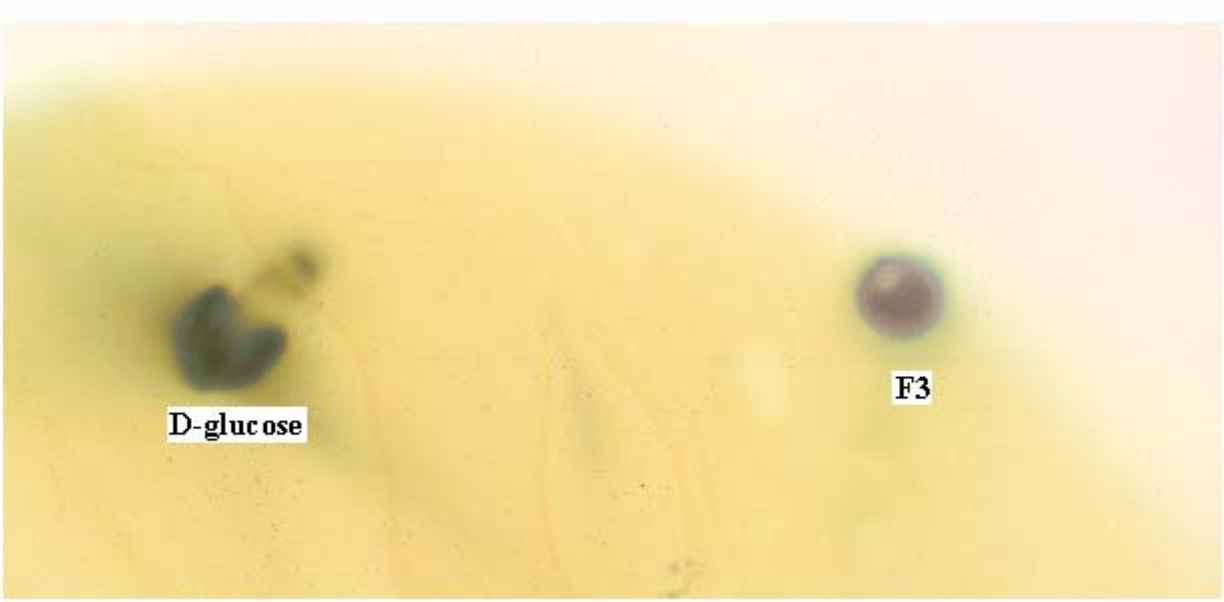


(A) et **(B)**. Silver-stained SDS-PAGE (15 % gel) profiles F2 and F3 purified by anion exchange chromatography (final purified protein): 1.5 µg of protein was loaded.
(C). Coomassie Brilliant Blue R250 SDS-PAGE (12% gel) profile of F2 and F3 purified by anion exchange chromatography: 15 µl of each fraction was loaded on the gel.

2.3.3 Glycoprotein analysis by Thin Layer Chromatography

Glycoprotein analysis of the purified fraction is shown in Figure XII. A black burned spot in F3 indicates the presence of a glycosilated component

Figure XII: Glycoprotein component of purified fraction visualised on thin layer chromatography by spraying with orcinol.

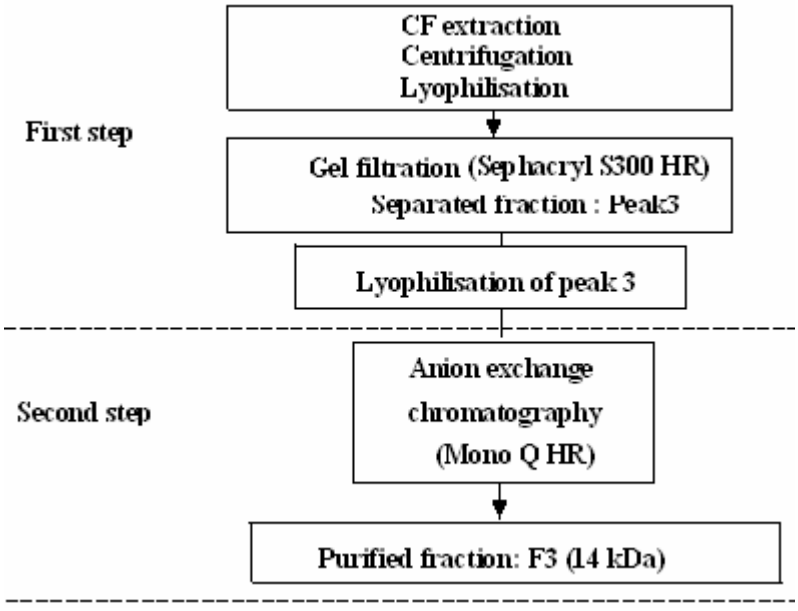


2.4 Discussion and conclusions

This study has shown that Sephacryl S-300 HR column (gel filtration) in combination with Mono Q HR column (anion exchange) can be used on HPLC to isolate a protein fraction with a major band of 14 kDa from *T. solium* cyst fluid. To obtain a purified fraction of 14 kDa from CF, the best purification strategy was the gel filtration followed by anion exchange chromatography (Figure XIII). Using Sephacryl S-200 gel filtration to purify *T. solium* extract, Cheng & Ko (1992) obtained three major peaks of proteins, but all of them contained a mixture of antigens with molecular weight ranging from 36 to 180 kDa. The difference observed between the fractions separated from CF by Sephacryl S-200 and Sephacrryl S-300 was either related to the difference between the 2 methods used to extract cyst fluid or to the resolution power of the two types of columns (Amersham Biosciences handbook).

The purified fraction seems to be a glycoprotein based on the positive signal following the orcinol spray. However, a better characterisation of the fraction is needed to confirm these results. A GP of 14 kDa has also been identified by Tsang *et al*. 1989 in a fraction isolated from cyst fluid with lentil-lectin affinity chromatography (LLGP). Greene *et al*. (1999) reported that the 14 kDa antigen may be a subunit of a larger group of antigenic proteins of *T. solium* cysticerci ranging from 25 to 45 kDa, purified by lentil-lectin affinity chromatography.

Figure XIII: Schematic diagram of the isolation of the 14 kDa fraction (F3) from crude cyst fluid of *T. solium* cysticerci



F3 was selected as purified antigen for further evaluation with Ab-ELISA

3 EVALUATION OF THE PURIFIED ANTIGEN FRACTION (F3)

ELISA's for detecting antibodies against metacestodes of *T. solium* have been developed by several authors. However, there are few tests that have both a high specificity and a high sensitivity (Dorny *et al*., 2000). Moreover cross-reaction with antibodies against other helminth parasites remains a challenge for test development in the immunodiagnosis of *T. solium* cysticercosis. The aim of this part of the study was to evaluate the diagnostic potential of a purified antigen fraction (F3) from *T. solium* cysticerci obtained by 2-step high-performance liquid chromatography (HPLC) as described above. The fraction was used in an ELISA for antibody detection for the diagnosis of porcine cysticercosis. The diagnostic sensitivity and specificity of ELISA using F3 were determined by non-parametric Receiver Operating Characteristic (ROC) analysis (Zweig & Campbell, 1993).

3.1 Materials and methods

3.1.1 Analytic Sensitivity and Specificity of the Purified Fraction

The analytic sensitivity of the Ab-ELISA using F3 for antibodies detection is defined as the smallest detectable amount of antibodies in serum samples from pigs experimentally infected with different doses of *T. solium* eggs. The analytical specificity is defined as the degree to which the test does not cross-react with serum from pigs infected with other parasites (Jacobson, 1998).

- Standardisation of the ELISA using the purified fraction

The steps in optimisation of the different reagents and procedures of the test were adapted from the protocols described by Jacobson (1998) and Diouf (2000). Five reference sera, obtained at week 10 post infection (PI) from pigs experimentally infected with different doses of *T. solium* eggs (2 heavily, and 3 lightly infected pigs), were used as positive control sera in all the steps of standardisation. Four negative sera taken before infection of the same pigs were used as negative controls. The optimal dilutions of antigens, serum and conjugate were first determined by checkerboard titration.

- Serum samples for evaluation of F3 analytic sensitivity

Serum samples of the 5 previous pigs, experimentally infected with *T. solium* eggs (34 sera at different weeks post-infection) obtained from Cameroon, were used to determine the ability of the purified fraction to detect antibodies against *T. solium*. The test conditions of these experimental infections were described by Nguekam *et al.* (2003). Table I shows a summary of the infection status of the five pigs at necropsy:

Table I: Number and status of cysticerci in five pigs experimentally infected with different doses of *T. solium* eggs (Nguekam *et al*., 2003).

Pigs	Number of *T. solium* eggs administered	Total number of cysticerci recovered at necropsy	Satus of cysticerci		Ag-ELISA
			Viable	Caseous/calcified	
I-4	1000*	3	0	0/3	Negative
II-3	10000*	3	0	0/3	Negative
III-4	100000*	2	1	0/1	Positive
IV-1	Proglottids**	>3000/kg			Positive
IV-2	Proglottids**	>3000/kg			Positive

* Light infection; ** heavy infection

- Serum samples for evaluation of F3 analytic specificity

The potential cross reactivity of F3 was examined using sera of pigs and rabbits that received heterologous infections (Table II). These samples were obtained from the laboratory of the Department of Animal Health of the Institute of Tropical Medicine (Belgium). Negative serum samples from 16 Cameroonian pigs and serum samples from the above 5 experimentally infected pigs obtained at week 16 post infection were used as controls. The mean value of ELISA optical density of controls plus 3 standard deviations was taken as the cut-off.

Table II: Serum samples of pigs and rabbits infected with some common parasites

Sera of pigs infected with	**Number of serum samples**
Trichinella spiralis (EI)	3
Fasciola hepatica (NI)	2
Metastrongylus apri (EI)	2
Taenia hydatigena (NI)	10
Taenia s. asiatica (EI)	8
Trypanosoma congolense (EI)	6
Sarcoptes scabiei (NI)	5
Sera of rabbits hyper-immunised with	
CF of Taenia solium	1
CF of Taenia hydatigena	1

EI: experimental infection
NI: Natural infection

For the serum of hyper-immunised rabbits, the standardised ELISA was similar to that used for pigs, but with some minor modifications: conjugate dilution (anti-rabbit IgG), 1/20,000; blocking buffer, PBS-Tween20- 2% NBCS.

3.1.2 Diagnostic sensitivity and specificity of the purified fraction

The diagnostic sensitivity was defined as the proportion of pigs infected with *T. solium* cysticerci identified as positive by the test, and the diagnostic specificity was defined as the proportion of non-infected pigs identified as negative by the test.

- Serum samples for diagnostic evaluation of F3

Zambian serum samples:

Sixty-six reference serum samples of pigs originating from Zambia (38 non- infected pigs and 28 pigs harboring cysticerci at carcass dissection) were obtained from the serum bank of the Department of Animal Health of the Institute of Tropical Medicine. The pigs had been randomly purchased from villages with endemic porcine cysticercosis and, following slaughter, half of the carcass had been sliced in such a way that all fully developed cysts could be revealed and enumerated (Dorny *et al*., 2004b). All sera had previously been tested for i) the presence of circulating antigens using a monoclonal based sandwich ELISA ii) the presence of antibodies using an ELISA with *T. crassiceps* antigen. The status of pigs at carcass dissection is given in Table III.

Table III: Status of 66 Zambian village pigs at carcass dissection.

Test	Status	Number of carcasses	Number of pigs with viable *T. solium* cysticerci		Only degenerated cysticerci
			Light infection	moderate/heavy infection	
Carcass	Positive	28	10	12	6
inspection	Negative	38	0	0	0

Cameroonian serum samples:

Fifty-eight serum samples of pigs originating from Cameroon (28 pigs from cysticercosis -free farms and 30 pigs with cysticerci at tongue inspection (Pouedet *et al*., 2002)) were also obtained from the serum bank of the Department of Animal Health of the Institute of Tropical Medicine.

-Nonparametric ROC analysis

The diagnostic ability of the ELISA test was determined with nonparametric receiver operating characteristic (ROC) analysis (StataCorp, 2001. Stata Statistical Software, Release 7.0. Stata Corporation 2001, College Station, TX). The ROC methodology is appropriate in a situation where there are 2 possible true disease stages (diseased /normal). The sensitivity of the test is depicted on the Y-axis, whereas de X-axis shows the false positive (1-specificity). A diagonal line in a plot corresponds to a test that is positive or negative just by chance.

- Cut-off selection

To choose a realistic cut-off, ELISA results ranging from the lower to the upper OD value were plotted in a two-graph ROC (TG-ROC) curve as described by Greiner *et al*. (1995). In a TG-ROC, a test's sensitivity and specificity (y-axis) are plotted for each cut-off (x-axis). The cut-off where sensitivity and specificity are equal (Se = Sp) was set as the intersection point of the two graphs and was also selected as the cut-off for discrimination between the positive and negative serum samples.

3.2 Results

3.2.1 Analytic sensitivity

-Standardisation of Ab-ELISA-F3:

The definitive protocol of ELISA using the F3 antigen for detection of cysticercosis is summarised in table IV.

Table IV: Standardised ELISA protocol

Steps	Reagent	Dilution	Volume/well	Incubation
Coating	Antigen F3	0.5 µg of F3 in 1 ml of Carbonate buffer (0.06 M, pH 9.6)	100 µl	1 hour at 37 °C followed by 1 night at 4°C
1 Washing*				
Blocking	PBS-Tw20+ 2% gelatine		150 µl	1 hour at 37 °C on shaker
Sera	Serum samples	1/500 in PBS-Tw20-0.1% gelatine-EDTA 5 mM	100 µl	1 hour at 37 °C on shaker
5 washings				
Conjugate	Anti- pig IgG Peroxidase	1/30,000 in PBS-Tw20-0.1% gelatine-EDTA 5 mM	100 µl	1 hour at 37 °C on shaker
5 washings				
Substrate	TMB peroxidase substrate and Peroxidase Solution B (H_2O_2) (KPL, Gaithersburg, USA)	Equal volume of TMB and solution B	100 µl	5-10 min at room temperature
Stop solution	Phosphoric acid (H_3PO_4)	1 M	100 µl	
Read the plate at 450 nm with reader (Multiscan EX, Termo Labsystem)				

* Washing buffer: Phosphate buffer saline-Tween 20 (0.05%) (PBS-T20)

-Comparison of analytic and diagnostic characteristics of Ab-ELISA-CF and Ab-ELISA-F3 using serum samples of experimentally infected pigs

The results of the ELISA's with CF and F3 as antigens for detecting antibodies in serum samples of experimentally infected pigs are shown in Figure XIV. The absorbency values of serum samples from heavily infected pigs were higher compared with those of lightly infected pigs using CF as antigen. In contrast, with the use of F3 there was an important rise in antibody titres in lightly infected pigs between week 2 and week 4 PI. For all animals, the antibody activity (optical density) in F3 decreased to control levels 2 or 3 months PI. The optical densities persisted at high level throughout experiment in CF for heavily infected animals, but all lightly infected pigs became negative at necropsy.

3.2.2 Evaluation of cross-reactions in the Ab-ELISA-CF and Ab-ELISA-F3

Figure XV shows the results of the ELISA's with CF and F3 as antigens in which the reactivity of serum samples from pigs infected with other parasites was assessed. No cross-reactions were recorded with 36 serum samples from pigs infected with other parasites using the F3 antigen. With CF, cross-reactions were observed with some sera of pigs, naturally infected with *T. hydatigena* cysticerci, *Fasciola hepatica* and *Metastrongylus apri.* No antibodies were detected with F3 antigen in serum of rabbits that were hyper-immunized with crude cyst fluid of *T. hydatigena* metacestodes (Figure XVI).

Figure XIV: Comparison of kinetics of circulating antibodies detected by CF and F3 antigens in serum samples of lightly and heavily *T. solium* cysticerci infected pigs.

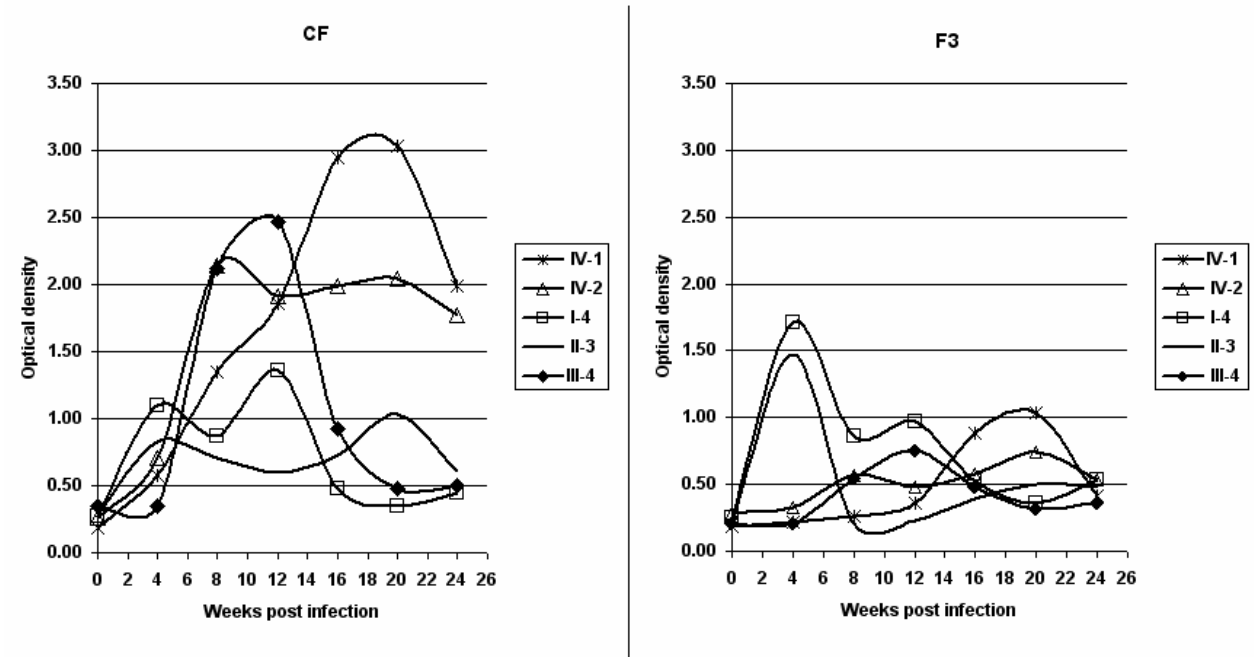


Figure XV: Testing the cross-reactivity in the Ab-ELISA-CF and Ab-ELISA-F3 with serum samples of pigs infected with other parasites than *T. solium*.

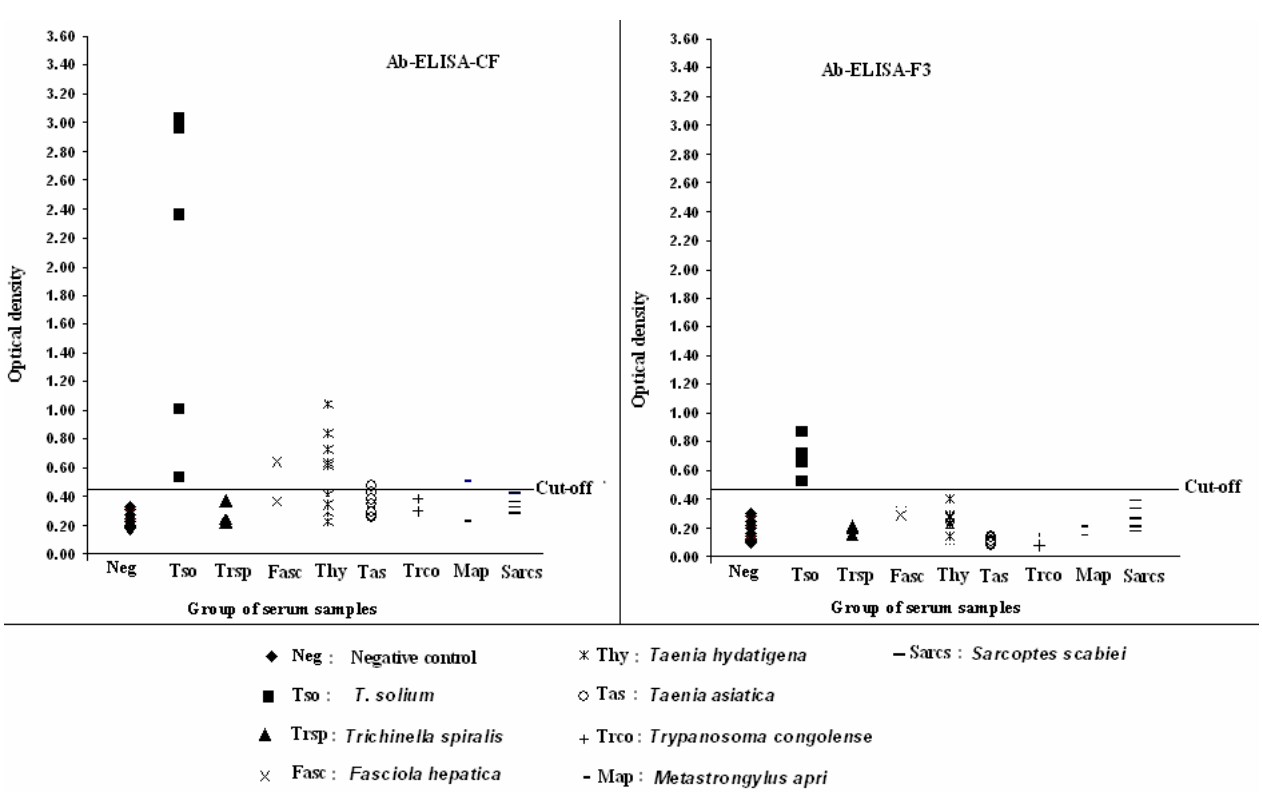
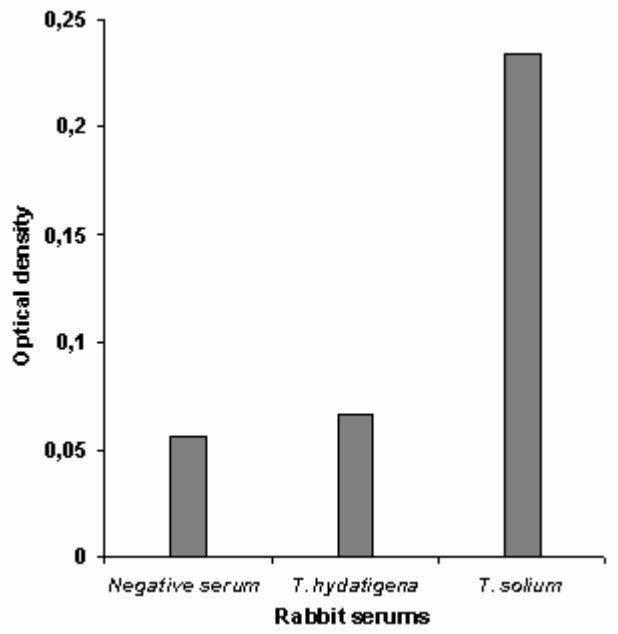


Figure XVI: Testing the cross-reactivity of the F3 fraction using polyclonal rabbit antiserum against *T. hydatigena* CF



3.2.3 ROC analysis using serum samples from Zambia

The ROC curves and area under the curve (AUC) for Ab-ELISA-CF and Ab-ELISA-F3 are shown in Figure XVII. The AUC can be interpreted as the probability that the ELISA result of a randomly drawn sample event is correct with regard to the infection status. Highly discriminatory tests have ROC curves that crowd toward the upper left corner with AUC approaching 1. It is clear from the Figure XVII that the AUC for CF (0.69) is higher than for F3 (0.48).

- Ab-ELISA-CF and Ab-ELISA-F3 cut-off selection and comparison of results to carcass dissection and Ag-ELISA.

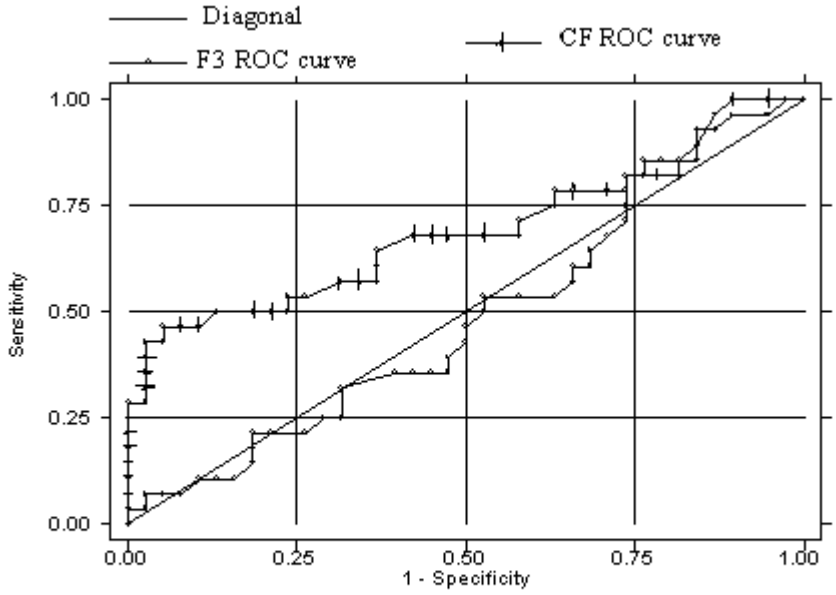
The sensitivity and specificity at each optical density obtained with the 66 serum samples are plotted in a two-graph ROC (Figure XVIII). The selected cut-off is the optical density at which Sensitivity (Se) and Specificity (Sp) are equal (intersection) both for CF and F3. For CF, Se and Sp intersect at a cut-off of 0.43 with Se=Sp= 62.16 %. For F3, the cut-off at the intersection of Se and Sp is 0.66 with Se=Sp= 50 %. This means that obtaining a Se much greater than 62 % or 50 % entails accepting quite a low Sp (and vice versa), respectively with CF and F3.

The comparison of the Ab-ELISA-F3 results with the results obtained by dissection and Ab-ELISA-CF is shown in Table V. With the Ab-ELISA-F3, 8 of 12 (67%) heavily infected pigs were positive, while 4 of 10 lightly infected pigs (40 %) and 3 of 6 pigs harboring only calcified cysts were positive. Within the group of pigs found negative at dissection, 20 (53 %) were positive (False positives). All the heavily infected pigs were positive with Ab-ELISA-CF (100%). Ab-ELISA-CF detected 3 of 10 lightly infected (30 %) and 3 of 6 pigs harbouring only calcified cysts, while 14 of 38 negative pigs at dissection were positive.

Table V: Comparison of the results of dissection with Ab-ELISA-CF and Ab-ELISA-F3 applied on 66 Zambian pigs

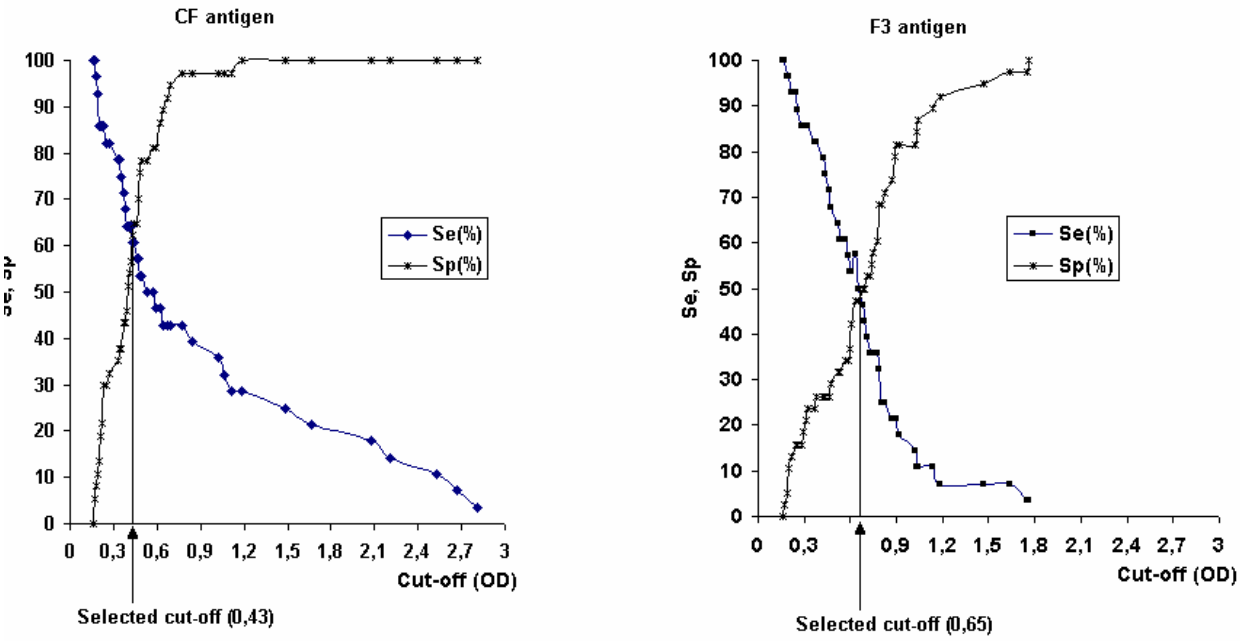
Number of samples	Carcass dissection	Ab-ELISA-CF	Ab-ELISA-F3	Light infection (1-100 cysts)	Heavy infection (>100 cysts)	Only calcified cyst
16	-	-	-	0	0	0
8	-	-	+	0	0	0
12	-	+	+	0	0	0
2	-	+	-	0	0	0
8	+	-	-	5	0	3
2	+	-	+	2	0	0
5	+	+	-	1	4	0
13	+	+	+	2	8	3

Figure XVII: Comparison of nonparametric ROC curves of ELISA's using CF and F3 as antigen on 66 reference serum samples from Zambian pigs



CF area under curve (AUC): 0.69
F3 area under curve (AUC): 0.48

Figure XVIII: Sensitivity (Se) and Specificity (Sp) of Ab-ELISA-CF and Ab-ELISA-F3 plotted against different cut-off levels obtained on 66 Zambian pigs.



3.2.4 ROC analysis using serum samples from Cameroonian pigs

Figure XIX shows the Ab-ELISA-CF and Ab-ELISA-F3 ROC curves calculated on the data of the documented Cameroonian pigs. The area under the curve (AUC) for both antigens is similar (0.81 and 0.78, respectively for CF and F3). The Se and Sp at selected cut-off (intersection of Se and Sp) are shown in Figure XX. For CF, Se and Sp intersect at a cut-off of 0.5 with Se=Sp= 71 %. For F3, the cut-off at the intersection of Se and Sp is 0.31 with Se=Sp= 68 %.

Figure XIX: Comparison of non-parametric ROC curve for ELISA using CF and F3 as antigen on 58 serum samples from Cameroonians pigs

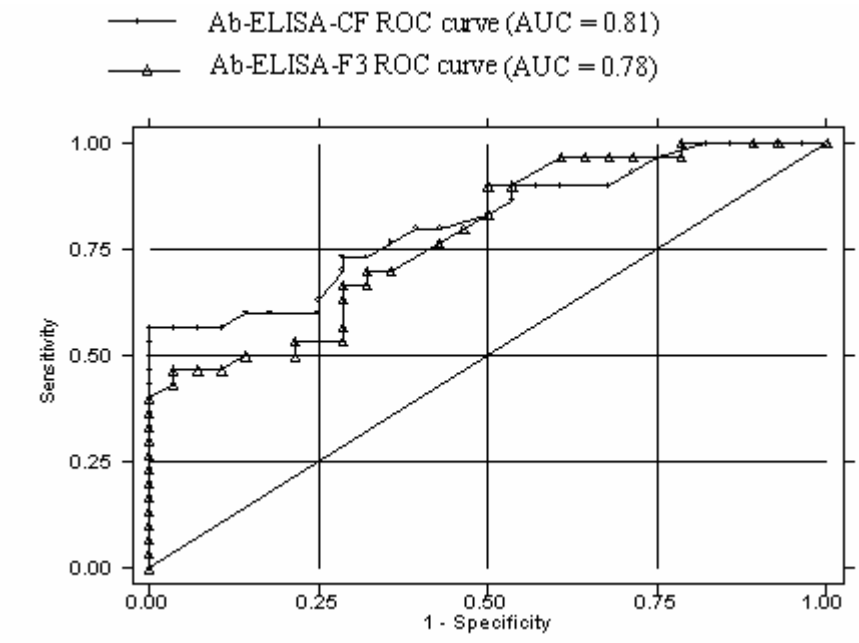
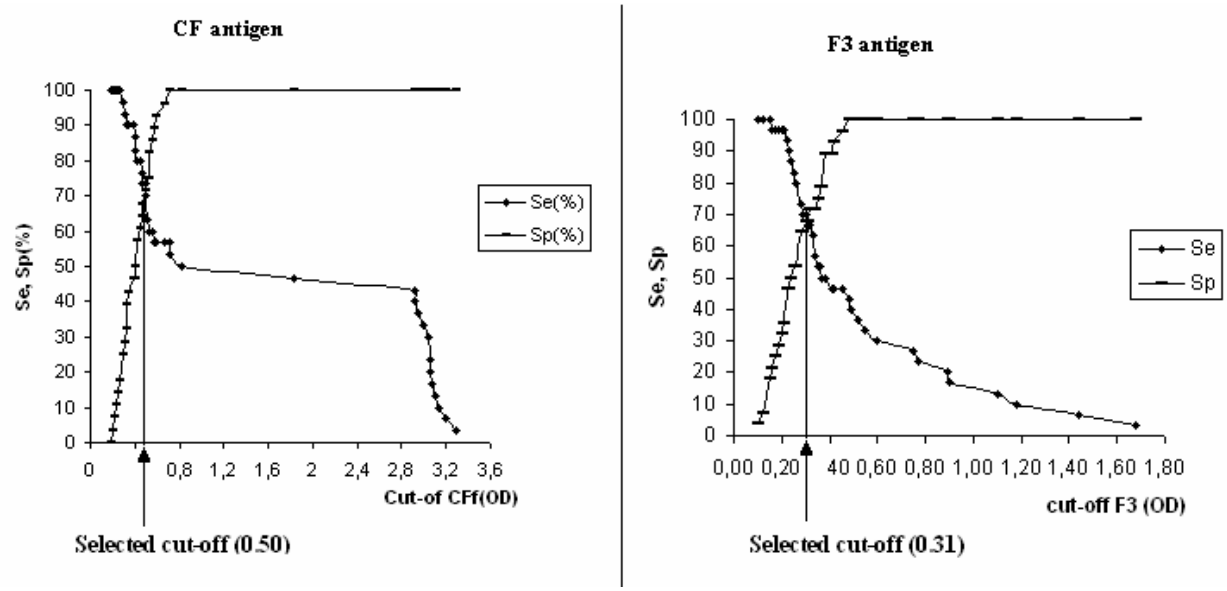


Figure XX: Sensitivity (Se) and Specificity (Sp) of Ab-ELISA-CF and Ab-ELISA-F3 plotted against different cut-offs obtained on 58 serum samples from Cameroonian pigs.



3.3 Discussion and conclusions

The crude antigens of *T. solium* cysticerci are cross-reactive and recognised by sera of pigs infected with *T. hydatigena* (Cheng & Ko, 1991). In the present study, we observed no cross-reaction of the 14 kDa fraction purified by gel filtration chromatography in combination with anion exchange chromatography. No cross-reaction was also recorded with serum samples from pigs infected with *T. s. asiatica*. This finding emphasises that the F3 antigen is a putative candidate for the diagnosis of *T. solium* cysticercosis in areas where *T. solium*, *T. hydatigena* and *T. asiatica* are present. However the species-specificity of this purified fraction should be further examined by testing the cross-reactivity with the other species of *Taenia.*

We used a non-parametric ROC analysis to evaluate the diagnostic sensitivity and specificity of F3 in an Ab-ELISA. To our knowledge, this methodology has not yet been used in the validation of tests for diagnosis of *T. solium* cysticercosis despite the fact that it is widely accepted as a fundamental concept in the process of test evaluation (Zweig & Campbell, 1993). In this study we found a low specificity for the Ab-ELISA-F3 (≤50%) using serum samples from a non-infected population originating from an endemic area. However, with a non-infected population selected from farms in area's where pigs had no previous contact with *T. solium* eggs (i.e. the Cameroonian serum samples), the diagnostic specificity increased (≤70%). Similar observations were reported in the case of diagnosis of human cysticercosis (Larralde et *al*. 1986). This observation confirms the tenet that the sensitivity and specificity values of a test are related to characteristics of a population to which it is applied (Greiner and Gardner, 2000).

4 BAYESIAN ROC ANALYSIS APPROACH AND ITS COMPARISON WITH THE CLASSICAL NON-PARAMETRIC ROC ANALYSIS FOR THE DIAGNOSTIC EVALUATION OF THE AB-ELISA-F3

4.1 Introduction

In this study, we developed a Bayesian approach for ROC analysis and compared it to the conventional non-parametric ROC analysis. Bayes' theorem provides the basis for obtaining an updated belief (posterior) about an existing hypothesis (prior) given new data (likelihood). It is the fundamental law of logical, rational thinking based on probability (Malakoff, 1999; Greenland, 1998). Bayes' theorem can be written formally as follows: Pr (H/Data) = Pr(Data/H) x Pr(H)/Pr(Data). The equation indicates that the probability (Pr) of an hypothesis (H) given the data equals the probability of the data, given the original hypothesis was correct, multiplied by the probability of the hypothesis before the data were obtained and divided by the average probability of the data. To simplify this concept for data analysis, the prior information (expert opinion) is given in the form of a beta distribution (Lawrence et *al*., 1995; Ouedraogo, 2001; Congdon 2003). The posterior opinion (updated belief) is proportional to the product of the likelihood and the beta distribution. In veterinary clinical practice and research, the data could be the results of a diagnostic test, a clinical trial, a survey, a laboratory experiment or a risk factor (Gardner, 2002). Bayesian methods were developed for estimating tests sensitivity and specificity and the disease prevalence for one or more tests applied to one or more populations (Enøe *et al*., 2000; Adel, 2002, Dorny et *al*., 2004b). To our knowledge, this is the first report on the development of a Bayesian ROC model based on multinomial distribution including all possible interactions between tests over all spectrum of cut-off (optical densities) obtained on disease and non disease population.

4.2 Material and methods

4.2.1 Data

-Ag-ELISA, Ab-ELISA-CF and Ab-ELISA-F3 results obtained from 66 Zambian pigs (Appendix 1):
The results obtained previously with Ag-ELISA, Ab-ELISA-CF and Ab-ELISA-F3 applied on 66 serum samples from Zambian pigs were used to validate the ROC model in conditional dependence between the tests.
-Results obtained with tongue inspection and Ab-ELISA-F3 applied on Cameroonian pigs serum samples are also used for ROC model in conditional independence between the tests (Appendix 2).

4.2.2 The model description

A Bayesian ROC approach based on multinomial distribution and conditional (in-) dependence between multiple tests was developed and run on WinBUGS 1.4 (Spiegelhalter *et al*., 2003). Gardner *et al*. (2000) and Adel (2002) described the conditional dependence between multiple tests in detail. Briefly in the case of 3 tests, there are 15 parameters to be estimated. These parameters are:

1) the prevalence;
2) the sensitivity and specificity of the first test
3) two conditional sensitivities and two conditional specificity's for the second test
4) Four conditional sensitivities and four conditional specificity's for the third test

In a Bayesian ROC model, based on three tests, the number of the parameters to be estimated is given by the following formula: N = 14*n+1 where N= number of the parameters; n= number of possible cut-off of ELISA optical densities. It means that there are 14 parameters with variable values following the changes in cut-off (optical density) while the prevalence of the disease remains constant. The model description in the case of conditional dependence between 3 tests for Bayesian ROC analysis is given in appendix 3. With a conditional independence model, one sensitivity and one specificity are estimated for each test at each cut-off (Appendix 4).

4.3 Results

4.3.1 Bayesian ROC analysis approach and its comparison with the non-parametric ROC analysis for diagnostic evaluation of the Ab-ELISA-F3

Figure XXI and Figure XXII show the non-parametric ROC and Bayesian ROC curves using serum samples from Zambian and Cameroonian pigs, respectively. The ROC curves obtained by the Bayesian approach and non-parametric ROC analysis are identical. The appendices 3.3 and 4.2 show the values of sensitivity and specificity at each cut-off (optical density) both for Bayesian ROC and non-parametric ROC analysis obtained with Zambian and Cameroonian pig serum samples.

Figure XXI: Comparison of non-parametric and Bayesian ROC curves of the Ab-ELISA-F3 using 66 serum samples of Zambian pigs.

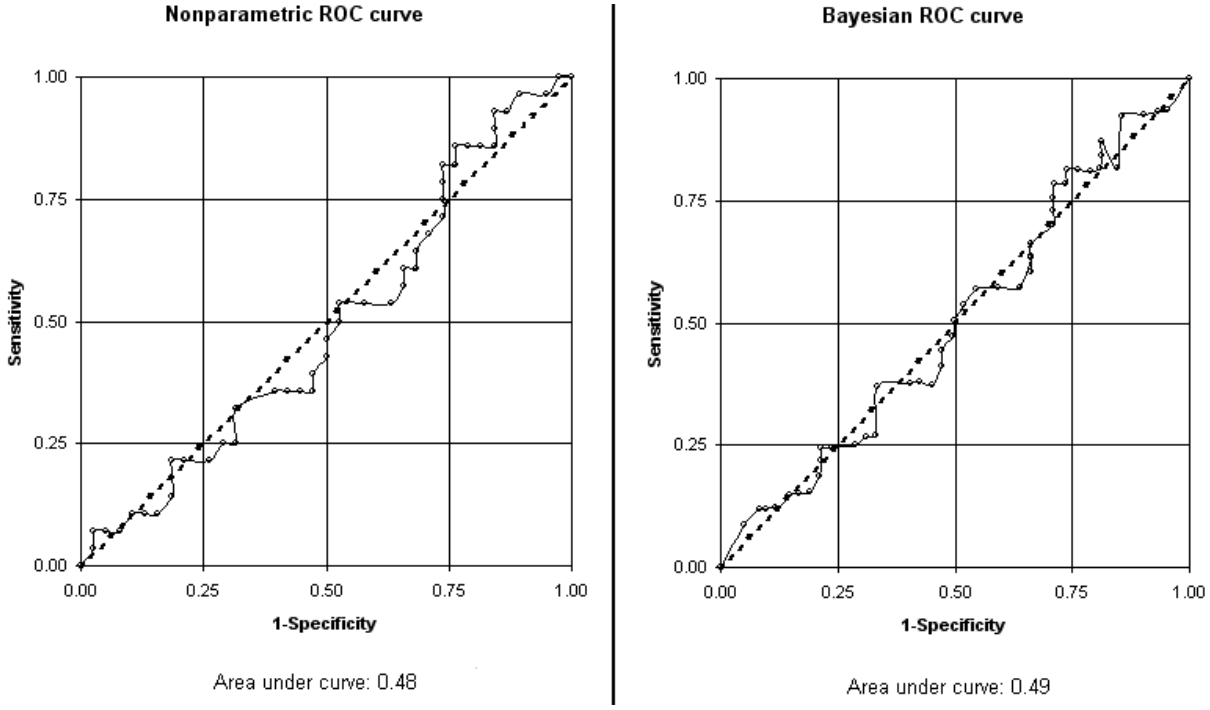
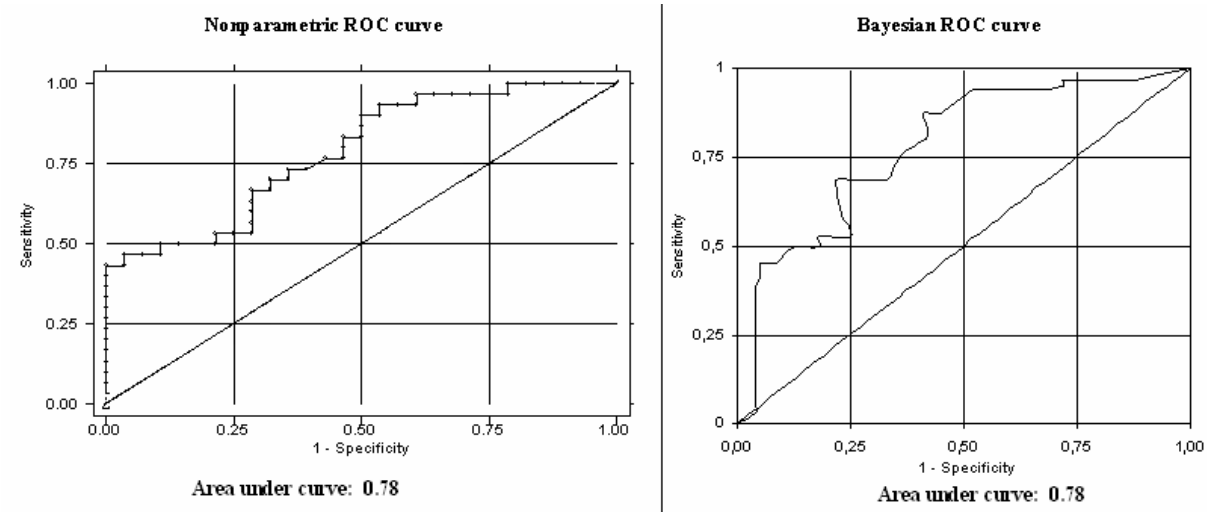


Figure XXII: Comparison of the non-parametric and Bayesian ROC curves of the Ab-ELISA-F3 using 58 serum samples of Cameroonian pigs



4.4 Discussion and conclusions

In a classical non-parametric ROC analysis, all possible combinations of diagnostic sensitivity and specificity can be achieved by changing the test's cut-off value (Zweig & Campbell, 1993; Greiner *et al*., 2000). This means that the diagnostic sensitivity and specificity are a function of the selected cut-off value. A Bayesian approach can be used to estimate the diagnostic sensitivity and specificity by combining expert opinion with experimental data (Enoe *et al*., 2000, Dorny *et al*., 2004b). In this study, we combined the concept of a ROC analysis and the Bayesian methodology to develop a Bayesian ROC analysis. The results obtained with the non-parametric ROC analysis and the Bayesian ROC model were highly similar. The non-parametric ROC analysis is generally used to select the optimum cut-off value to allow discrimination between diseased and non-diseased animals (Dohoo *et al*., 2003).

Because the classical non-parametric ROC and the Bayesian ROC analysis are similar, it is possible to select a posterior cut-off based on expert opinion and experimental data. Another advantage of the Bayesian method developed in this study is that, several tests could be combined to select the optimal cut-off.

This finding is promising for diagnostic test evaluation, but more investigations are needed to validate the usefulness of the model.

5 GENERAL DISCUSSION AND CONCLUSIONS

5.1 General discussion

Glycoproteins (GP) of *T. solium* metacestode extracts have been characterised and showed evidence of specificity for the diagnosis of *T. solium* cysticercosis (Grogl *et al,* 1985). Furthermore, several studies have demonstrated that the 10-26 kDA GP components of cysticerci are the ones showing the highest specificity for serodiagnosis of cysticercosis (Gottstein *et al*., 1986; Tsang *et al*., 1989; Yang *et al*., 1998; Ito *et al*., 1998). The purpose of this work was to isolate *T. solium*-specific antigens with a molecular weight between 10 and 26 kDa from crude *T. solium* cyst fluid. Using Sephacryl S-300 gel filtration in combination with anion exchange in a HPLC system, two protein fractions with major bands of 14 kDA and 14-20 kDa on SDS-Page, were isolated. However, the fraction showing a major band of 14 kDa was considered in this work as the most purified fraction (F3).

In an ELISA using F3 as antigen (Ab-ELISA-F3), antibodies were detected between week 2-6 post infection, both in serum samples of heavily and lightly infected pigs. Using GP's purified by iso-electric focusing as antigen, Sato *et al*. (2003) observed that the antibody response of heavily and lightly infected pigs started 30 and 60 days after infection, respectively. The earlier detection of antibodies to F3 as compared with GP's purified by iso-electric focusing suggests that the Ab-ELISA-F3 has a good analytic sensitivity (Jacobson, 1998). The optical densities with F3 were higher in lightly infected pigs than in heavily infected pigs between week 2 and week 6 after infection. This difference is difficult to explain, but may be related to the infection dose. It is known that very high doses of antigen might induce tolerance of the immune response (Tizard, 1992), which could explain the lower antibody titres in the heavily infected animals. However, for all animals in this study, antibody levels to F3 decreased to pre-infection levels between 2 or 3 months after their appearance. F3 might be an antigen expressed early during oncosphere development, and hence, inducing an early antibody response. If so, it might be a good marker for the establishment of cysticerci and the Ab-ELISA-F3 could be useful in epidemiological studies to measure the incidence of *T. solium* cysticercosis by the detection of recently infected animals.

In this study we used a non-parametric ROC analysis to evaluate the diagnostic potential of the Ab-ELISA-F3. In practice, cut-off values of ELISA's and other quantitative serodiagnostic tests are often established as the mean plus two-fold standard deviation of the results observed with the sera from a negative reference population (Richardson *et al*., 1983). Barajas-Rojas *et al.* (1993) pointed out that this approach theoretically leads to a specificity of 97.5%. The procedure is clearly not adequate if the test values follow a skewed or multinomial distribution. Cut-off values can also be calculated by comparing the optical density of each sample with the mean of a series of eight sera (Dorny *et al*., 2000). This assumption only holds true if the sample is represented by only a single variable, so it does not contribute to the degrees of freedom or to the estimate of the variance within groups (Sokal and Rohlf, 1981). In many applications of serodiagnostic tests it may be more appropriate to select cut-off values with equal weights on Se and Sp (Greiner *et al*., 1995). Receiver operating characteristic (ROC) analysis (Zweig and Campbell, 1993) can describe these two parameters as a function of the selected cut-off. It is, however, not possible to read any cut-off value for a selected combination of Se and Sp directly from a ROC curve. Therefore, a two-graph ROC curve described by Greiner *et al*. (1995) provides a visualisation of the two test parameters as function of the cut-off.

With the use of 66 reference serum samples from naturally infected Zambian pigs, in which the total number of cysticerci were determined by dissection (28 pigs harboured *T.*

solium cysticerci and 38 were negative at dissection), we found that the Ab-ELISA-F3 had low diagnostic specificity values. More than 50 % of negative pigs were false positive at the selected cut-off. It can, however not be excluded that a pig, not infected with cysticerci, has specific antibodies against the F3 antigen. One reason for this observation is that some pigs living in endemic cysticercosis areas may develop a transient serologic antibody response specific for F3 antigen. This phenomenon was recently reported in human cysticercosis by Garcia *et al*. (2001). On the other hand, as assumed previously, the negative pigs at dissection which were positive with Ab-ELISA-F3, may have developing oncospheres which can not be detected at dissection. Using serum samples from Cameroonian pigs in which the non infected group were pigs originating from an area free of *T. solium* and the infected group were classified by tongue inspection, we found higher diagnostic sensitivity and specificity values compared to serum samples from Zambia. This difference confirms that the sensitivity and specificity of a test vary with characteristics of the population to which they are applied (Greiner & Gardner, 2000). Therefore, it is important to know which characteristics of a population affect these two parameters. Because a ROC curve plots the sensitivity versus the false positive rate (1-Specificity) of a test to select an optimal cut-off to distinguish between infected and non-infected animals, the use of a population from an endemic cysticercosis area could lead to a lower diagnostic specificity at any selected cut-off. A similar observation was reported in the diagnosis of brucellosis: the specificity of a serologic test for *Brucella abortus* is higher when the test is used in a non-vaccinated population (Dohoo *et al.*, 2003). The absence of antibodies (no immune response against *Brucella abortus)* may explain this difference. However, another drawback of our study is the low sample size, which in turn might influence the outcome of the ROC analysis.

In addition to the 'traditional' ROC analysis, we also developed a Bayesian ROC analysis for evaluation of a diagnostic test. In the last decade there has been an increasing application of the Bayesian methodology in veterinary sciences, especially in the areas of test validation and prevalence estimation (Enøe, 2000; Dorny *et al*., 2004b). In this study, the pertinence of the use of Bayesian ROC analysis was shown by the fact that results from several tests could be combined to estimate the diagnostic sensitivity and specificity of the new test in a conditional (in-) dependence model. Hereto, the complete spectrum of optical densities of the new test were analysed by WinBUGS (Spiegelhalter *et al*., 2003). The results obtained by non-parametric ROC analysis and Bayesian analysis were similar. This observation confirmed the validity of the Bayesian model. This finding is interesting because with the Bayesian ROC analysis, it is possible to select a posterior cut-off to estimate posterior sensitivity and specificity.

5.2 General conclusions

This study has shown that HPLC using sephacryl S-300 gel filtration can be used to fractionate crude fluid of *T. solium* cysticerci in three major absorbing peaks. SDS-PAGE showed the second and third peaks contained only low major proteins band of 14-20 kDa and 14 kDa, respectively. In a second step purification in which the three peaks were individually purified by HPLC using anion exchange chromatography, the third peak gave the most purified fraction (F3).

Ab-ELISA using F3 detected antibodies before the first month of experimental post-infection in both lightly and heavily infected pigs. In addition, no cross-reaction was observed with the other parasites used in this study. This observation means that F3 has a good analytic sensitivity and specificity (Jacobson, 1998). However the species-specificity of this purified fraction should be further examined by testing the cross-reactivity with the other species of *Taenia.*

The diagnostic performance of this fraction was less satisfying with 66 reference serum samples from Zambia.

However, the species-specificity of this antigen makes it a putative candidate for the production of monoclonal or polyclonal antibodies that could be used in ELISA's for the detection of circulating antigens in infected pig. Alternatively, the Ab-ELISA-F3 could be combined with other diagnostic tests in epidemiological surveys of porcine cysticercosis.

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7 APPENDICES: BAYESIAN ROC ANALYSIS MODEL

Appendix 1: Agreement between the three different tests for detection of cysticercosis in Zambian dissected pigs over a range cut-off values from the lower to the upper optical densities.

Cut-off (optical density)	No. of samples (n=66)	Tests		
		Ag-ELISA	Ab-ELISA-CF	Ab-ELISA-F3
	0	-	-	-
	19	-	-	+
≥0.16	0	-	+	-
	13	-	+	+
	0	+	-	-
	15	+	-	+
	0	+	+	-
	19	+	+	+
≥0.17	0	-	-	-
	19	-	-	+
	0	-	+	-
	13	-	+	+
	1	+	-	-
	14	+	-	+
	0	+	+	-
	19	+	+	+
≥0.19	0	-	-	-
	19	-	-	+
	0	-	+	-
	13	-	+	+
	3	+	-	-
	12	+	-	+
	0	+	+	-
	19	+	+	+
≥0.20	1	-	-	-
	18	-	-	+
	0	-	+	-
	13	-	+	+
	4	+	-	-
	11	+	-	+
	0	+	+	-
	19	+	+	+
≥0.22	2	-	-	-
	17	-	-	+
	0	-	+	-
	13	-	+	+
	5	+	-	-
	10	+	-	+
	0	+	+	-
	19	+	+	+

≥0.25	3	-	-	-
	16	-	-	+
	0	-	+	-
	13	-	+	+
	5	+	-	-
	10	+	-	+
	0	+	+	-
	19	+	+	+
≥0.26	3	-	-	-
	16	-	-	+
	0	-	+	-
	13	-	+	+
	6	+	-	-
	9	+	-	+
	0	+	+	-
	19	+	+	+
≥0.28	4	-	-	-
	15	-	-	+
	0	-	+	-
	13	-	+	+
	6	+	-	-
	9	+	-	+
	0	+	+	-
	19	+	+	+
≥0.29	5	-	-	-
	14	-	-	+
	0	-	+	-
	13	-	+	+
	6	+	-	-
	9	+	-	+
	0	+	+	-
	19	+	+	+
≥0.31	5	-	-	-
	14	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	0	+	+	-
	19	+	+	+
≥0.32	6	-	-	-
	13	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	0	+	+	-
	19	+	+	+
≥0.37	7	-	-	-
	12	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	0	+	+	-
	19	+	+	+

≥0.38	8	-	-	-
	11	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	0	+	+	-
	19	+	+	+
≥0.43	8	-	-	-
	11	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	1	+	+	-
	18	+	+	+
≥0.44	9	-	-	-
	10	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	1	+	+	-
	18	+	+	+
≥0.46	9	-	-	-
	10	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	2	+	+	-
	17	+	+	+
≥0.47	9	-	-	-
	10	-	-	+
	0	-	+	-
	13	-	+	+
	7	+	-	-
	8	+	-	+
	4	+	+	-
	15	+	+	+
≥0.52	10	-	-	-
	9	-	-	+
	0	-	+	-
	13	-	+	+
	8	+	-	-
	7	+	-	+
	4	+	+	-
	15	+	+	+
≥0.53	10	-	-	-
	9	-	-	+
	0	-	+	-
	13	-	+	+
	9	+	-	-
	6	+	-	+
	4	+	+	-
	15	+	+	+

≥0.57	10	-	-	-
	9	-	-	+
	0	-	+	-
	13	-	+	+
	8	+	-	-
	7	+	-	+
	4	+	+	-
	15	+	+	+
≥0.59	10	-	-	-
	9	-	-	+
	0	-	+	-
	13	-	+	+
	8	+	-	-
	7	+	-	+
	5	+	+	-
	14	+	+	+
≥0.6	11	-	-	-
	8	-	-	+
	0	-	+	-
	13	-	+	+
	8	+	-	-
	7	+	-	+
	6	+	+	-
	13	+	+	+
≥0.61	12	-	-	-
	7	-	-	+
	1	-	+	-
	12	-	+	+
	8	+	-	-
	7	+	-	+
	6	+	+	-
	13	+	+	+
≥0.63	13	-	-	-
	6	-	-	+
	1	-	+	-
	12	-	+	+
	9	+	-	-
	6	+	-	+
	6	+	+	-
	13	+	+	+
≥0.65	13	-	-	-
	6	-	-	+
	1	-	+	-
	12	-	+	+
	11	+	-	-
	4	+	-	+
	6	+	+	-
	13	+	+	+
≥0.68	13	-	-	-
	6	-	-	+
	1	-	+	-
	12	-	+	+
	12	+	-	-
	3	+	-	+
	7	+	+	-
	12	+	+	+

≥0.69	13	-	-	-
	6	-	-	+
	1	-	+	-
	12	-	+	+
	12	+	-	-
	3	+	-	+
	8	+	+	-
	11	+	+	+
≥0.71	13	-	-	-
	6	-	-	+
	2	-	+	-
	11	-	+	+
	13	+	-	-
	2	+	-	+
	8	+	+	-
	11	+	+	+
≥0.73	13	-	-	-
	6	-	-	+
	2	-	+	-
	11	-	+	+
	13	+	-	-
	2	+	-	+
	9	+	+	-
	10	+	+	+
≥0.74	12	-	-	-
	5	-	-	+
	2	-	+	-
	11	-	+	+
	13	+	-	-
	2	+	-	+
	12	+	+	-
	9	+	+	+
≥0.75	14	-	-	-
	5	-	-	+
	3	-	+	-
	10	-	+	+
	13	+	-	-
	2	+	-	+
	10	+	+	-
	9	+	+	+
≥0.78	14	-	-	-
	5	-	-	+
	3	-	+	-
	10	-	+	+
	13	+	-	-
	2	+	-	+
	11	+	+	-
	8	+	+	+
≥0.79	15	-	-	-
	4	-	-	+
	5	-	+	-
	8	-	+	+
	13	+	-	-
	2	+	-	+
	12	+	+	-
	7	+	+	+

≥0.80	15	-	-	-
	4	-	-	+
	5	-	+	-
	8	-	+	+
	14	+	-	-
	1	+	-	+
	13	+	+	-
	6	+	+	+
≥0.82	15	-	-	-
	4	-	-	+
	5	-	+	-
	8	-	+	+
	14	+	-	-
	1	+	-	+
	14	+	+	-
	5	+	+	+
≥0.87	15	-	-	-
	4	-	-	+
	5	-	+	-
	8	-	+	+
	15	+	-	-
	0	+	-	+
	15	+	+	-
	4	+	+	+
≥0.89	17	-	-	-
	2	-	-	+
	5	-	+	-
	8	-	+	+
	15	+	-	-
	0	+	-	+
	15	+	+	-
	4	+	+	+
≥0.90	18	-	-	-
	1	-	-	+
	5	-	+	-
	8	-	+	+
	15	+	-	-
	0	+	-	+
	15	+	+	-
	4	+	+	+
≥0.92	18	-	-	-
	1	-	-	+
	6	-	+	-
	7	-	+	+
	15	+	-	-
	0	+	-	+
	15	+	+	-
	4	+	+	+
≥1.02	18	-	-	-
	1	-	-	+
	6	-	+	-
	7	-	+	+
	15	+	-	-
	0	+	-	+
	16	+	+	-
	3	+	+	+

≥1.03	19	-	-	-
	0	-	-	+
	6	-	+	-
	7	-	+	+
	15	+	-	-
	0	+	-	+
	17	+	+	-
	2	+	+	+
≥1.04	19	-	-	-
	0	-	-	+
	7	-	+	-
	6	-	+	+
	15	+	-	-
	0	+	-	+
	17	+	+	-
	2	+	+	+
≥1.14	19	-	-	-
	0	-	-	+
	7	-	+	-
	6	-	+	+
	15	+	-	-
	0	+	-	+
	18	+	+	-
	1	+	+	+
≥1.18	19	-	-	-
	0	-	-	+
	8	-	+	-
	5	-	+	+
	15	+	-	-
	0	+	-	+
	19	+	+	-
	0	+	+	+
≥1.47	19	-	-	-
	0	-	-	+
	9	-	+	-
	4	-	+	+
	14	+	-	-
	1	+	-	+
	19	+	+	-
	0	+	+	+
≥1.64	19	-	-	-
	0	-	-	+
	10	-	+	-
	3	-	+	+
	14	+	-	-
	1	+	-	+
	19	+	+	-
	0	+	+	+
≥1.75	19	-	-	-
	0	-	-	+
	10	-	+	-
	3	-	+	+
	14	+	-	-
	1	+	-	+
	19	+	+	-
	0	+	+	+

≥1.76	19	-	-	-
	0	-	-	+
	12	-	+	-
	1	-	+	+
	15	+	-	-
	0	+	-	+
	19	+	+	-
	0	+	+	+

Appendix 2. Agreement between tongue inspection and Ab-ELISA-F3 at different cut-off levels obtained with 58 serum samples of Cameroonian pigs.

Cut-off (optical density)	No. of samples (n=58)	Tests	
		Tongue inspection	Ab-ELISA-F3
≥0.10	2	-	-
	26	-	+
	0	+	-
	30	+	+
≥0.12	3	-	-
	25	-	+
	0	+	-
	30	+	+
≥0.15	6	-	-
	22	-	+
	0	+	-
	30	+	+
≥0.16	6	-	-
	22	-	+
	1	+	-
	29	+	+
≥0.17	8	-	-
	20	-	+
	1	+	-
	29	+	+
≥0.19	9	-	-
	19	-	+
	1	+	-
	29	+	+
≥0.20	10	-	-
	18	-	+
	1	+	-
	29	+	+
≥0.21	11	-	-
	17	-	+
	1	+	-
	29	+	+
≥0.22	13	-	-
	15	-	+
	3	+	-
	27	+	+

≥0.23	14	-	-
	14	-	+
	3	+	-
	27	+	+
≥0.24	14	-	-
	14	-	+
	5	+	-
	25	+	+
≥0.25	15	-	-
	13	-	+
	6	+	-
	24	+	+
≥0.26	16	-	-
	12	-	+
	7	+	-
	23	+	+
≥0.28	18	-	-
	10	-	+
	9	+	-
	21	+	+
≥0.29	19	-	-
	9	-	+
	9	+	-
	21	+	+
≥0.30	19	-	-
	9	-	+
	9	+	-
	21	+	+
≥0.31	20	-	-
	8	-	+
	9	+	-
	21	+	+
≥0.32	20	-	-
	8	-	+
	10	+	-
	20	+	+
≥0.33	20	-	-
	8	-	+
	12	+	-
	18	+	+
≥0.34	20	-	-
	8	-	+
	14	+	-
	16	+	+
≥0.35	22	-	-
	6	-	+
	14	+	-
	16	+	+
≥0.36	22	-	-
	6	-	+
	15	+	-
	15	+	+
≥0.37	24	-	-
	4	-	+
	15	+	-
	15	+	+

≥0.38	25	-	-
	3	-	+
	16	+	-
	14	+	+
≥0.41	26	-	-
	2	-	+
	16	+	-
	14	+	+
≥0.42	27	-	-
	1	-	+
	16	+	-
	14	+	+
≥0.45	27	-	-
	1	-	+
	17	+	-
	13	+	+
≥0.48	28	-	-
	0	-	+
	18	+	-
	12	+	+
≥0.49	28	-	-
	0	-	+
	19	+	-
	11	+	+
≥0.52	28	-	-
	0	-	+
	20	+	-
	10	+	+
≥0.55	28	-	-
	0	-	+
	21	+	-
	9	+	+
≥0.6	28	-	-
	0	-	+
	22	+	-
	8	+	+
≥0.75	28	-	-
	0	-	+
	23	+	-
	7	+	+
≥0.77	28	-	-
	0	-	+
	24	+	-
	6	+	+
≥0.89	28	-	-
	0	-	+
	25	+	-
	5	+	+
≥0.90	28	-	-
	0	-	+
	26	+	-
	4	+	+
≥1.10	28	-	-
	0	-	+
	27	+	-
	3	+	+

≥1.18	28	-	-
	0	-	+
	28	+	-
	2	+	+
≥1.44	28	-	-
	0	-	+
	29	+	-
	1	+	+
≥1.68	28	-	-
	0	-	+
	30	+	-
	0	+	+

Appendix 3: Conditional dependence model for Bayesian ROC analysis

Assume that three tests (Ag-ELISA, Ab-ELISA-CF, Ab-ELISA-F3 so-called T1, T2, T3, respectively) are applied simultaneously on serum of individual animals of infected and non-infected groups of pigs, classified by carcass dissection, which was considered as the gold standard (Dorny *et al*., 2004b). The tests are based on similar biological processes (antibody or antigen detection); then these tests may be conditionally dependent (Gardner et al., 2000).
For one cut-off selected to estimate sensitivities, specificities and truth prevalence of the three, the number of parameters (conditional probabilities) to be estimated is 15 (Adel, 2002). The description of conditional probabilities is given as follows:

$Pr(D^+)$	p1 : prevalence
$Pr(T1^+/D^+)$	p2 : Sensitivity of the first test (se1)
$Pr(T1^-/D^-)$	p3 : Specificity of the first test (sp1)
$Pr(T2^+/D^+ \cap T1^+)$	p4
$Pr(T2^+/ D^+ \cap T1^-)$	p5
$Pr(T2^-/ D^- \cap T1^-)$	p6
$Pr(T2^-/ D^- \cap T1^+)$	p7
$Pr(T3^+/ D^+ \cap T1^+ \cap T2^+)$	p8
$Pr(T3^+/ D^+ \cap T1^+ \cap T2^-)$	p9
$Pr(T3^+/ D^+ \cap T1^- \cap T2^+)$	p10
$Pr(T3^+/ D^+ \cap T1^- \cap T2^-)$	p11
$Pr(T3^-/ D^- \cap T1^- \cap T2^-)$	p12
$Pr(T3^-/ D^- \cap T1^- \cap T2^+)$	p13
$Pr(T3^-/ D^- \cap T1^+ \cap T2^-)$	p14
$Pr(T3^-/ D^- \cap T1^+ \cap T2^+)$	p15

The sensitivities and specificities for the second and third tests are given as follows:
se2 =p2*p4+(1-p2)*p5
se3 = p2*(p4*p8+(1-p4)*p9)+(1-p2)*(p5*p10+(1-p5)*p11)
sp2 = p3*p6+(1-p3)*p7
sp3 = p3*(p6*p12+(1-p6)*p13)+(1-p3)*(p7*p14+(1-p7)*p15)

-In the case of two optical densities to be selected as cut-off, two possible sensitivities and two possible specificities could be estimated for each test. The truth prevalence of the disease remains constant. The number of parameters to be estimated is N= 14*2+1=29:

od1 : $Pr(D^+)$ p1
od1 : $Pr(T1^+/D^+)$ p2
od1 : $Pr(T1^-/D^-)$ p3
od1 : $Pr(T2^+/D^+ \cap T1^+)$ p4
od1 : $Pr(T2^+/ D^+ \cap T1^-)$ p5
od1 : $Pr(T2^-/ D^- \cap T1^-)$ p6
od1 : $Pr(T2^-/ D^- \cap T1^+)$ p7
od1 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^+)$ p8
od1 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^-)$ p9
od1 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^+)$ p10
od1 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^-)$ p11
od1 : $Pr(T3^-/ D^- \cap T1^- \cap T2^-)$ p12
od1 : $Pr(T3^-/ D^- \cap T1^- \cap T2^+)$ p13
od1 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^-)$ p14
od1 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^+)$ p15
od2 : $Pr(T1^+/D^+)$ p16
od2 : $Pr(T1^-/D^-)$ p17
od2 : $Pr(T2^+/D^+ \cap T1^+)$ p18
od2 : $Pr(T2^+/ D^+ \cap T1^-)$ p19
od2 : $Pr(T2^-/ D^- \cap T1^-)$ p20
od2 : $Pr(T2^-/ D^- \cap T1^+)$ p21
od2 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^+)$ p22
od2 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^-)$ p23
od2 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^+)$ p24
od2 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^-)$ p25
od2 : $Pr(T3^-/ D^- \cap T1^- \cap T2^-)$ p26
od2 : $Pr(T3^-/ D^- \cap T1^- \cap T2^+)$ p27
od2 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^-)$ p28
od2 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^+)$ p29

The probabilities (Pijk) of possible results with three tests at 2 possible cut-off values (od1, od2) are given as follows:
od1: P111 =p1*p2*p4*p8+(1-p1)*(1-p3)*(1-p7)*(1-p15)
od1: P110 = p1*p2*p4*(1-p8)+(1-p1)*(1-p3)*(1-p7)*p15
od1: P101 = p1*p2*(1-p4)*p9+(1-p1)*(1-p3)*p7*(1-p14)
od1: P100 = p1*p2*(1-p4)*(1-p9)+(1-p1)*(1-p3)*p7*p14
od1: P011 = p1*(1-p2)*p5*p10+(1-p1)*p3*(1-p6)*(1-p13)
od1: P010 = p1*(1-p2)*p5*(1-p10)+(1-p1)*p3*(1-p6)*p13
od1: P001 = p1*(1-p2)*(1-p5)*p11+(1-p1)*p3*p6*(1-p12)
od1: P000 = p1*(1-p2)*(1-p5)*(1-p11)+(1-p1)*p3*p6*p12
od2: P111 = p1*p16*p18*p22+(1-p1)*(1-p17)*(1-p21)*(1-p29)
od2: P110 = p1*p16*p18*(1-p22)+(1-p1)*(1-p17)*(1-p21)*p29
od2: P101 = p1*p16*(1-p18)*p23+(1-p1)*(1-p17)*p21*(1-p28)
od2: P100 = p1*p16*(1-p18)*(1-p23)+(1-p1)*(1-p17)*p21*p28
od2: P011 = p1*(1-p16)*p19*p24+(1-p1)*p17*(1-p20)*(1-p27)
od2: P010 = p1*(1-p16)*p19*(1-p24)+(1-p1)*p17*(1-p20)*p27
od2: P001 = p1*(1-p16)*(1-p19)*p25+(1-p1)*p17*p20*(1-p26)
od2: P000 = p1*(1-p16)*(1-p19)*(1-p25)+(1-p1)*p17*p20*p26
where i is the T1 result (1=positive, 0=negative), j is the T2 result (1=positive, 0=negative) and k is the T3 result (1=positive, 0=negative).

- In the case of three optical densities to be selected as cut-off, three possible sensitivities and three possible specificities could be estimated for each test. The true prevalence of the disease remains constant. The number of parameter to be estimated is N= 14*3+1=43:

od1 : $Pr(D^+)$	p1
od1 : $Pr(T1^+/D^+)$	p2
od1 : $Pr(T1^-/D^-)$	p3
od1 : $Pr(T2^+/D^+ \cap T1^+)$	p4
od1 : $Pr(T2^+/ D^+ \cap T1^-)$	p5
od1 : $Pr(T2^-/ D^- \cap T1^-)$	p6
od1 : $Pr(T2^-/ D^- \cap T1^+)$	p7
od1 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^+)$	p8
od1 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^-)$	p9
od1 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^+)$	p10
od1 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^-)$	p11
od1 : $Pr(T3^-/ D^- \cap T1^- \cap T2^-)$	p12
od1 : $Pr(T3^-/ D^- \cap T1^- \cap T2^+)$	p13
od1 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^-)$	p14
od1 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^+)$	p15
od2 : $Pr(T1^+/D^+)$	p16
od2 : $Pr(T1^-/D^-)$	p17
od2 : $Pr(T2^+/D^+ \cap T1^+)$	p18
od2 : $Pr(T2^+/ D^+ \cap T1^-)$	p19
od2 : $Pr(T2^-/ D^- \cap T1^-)$	p20
od2 : $Pr(T2^-/ D^- \cap T1^+)$	p21
od2 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^+)$	p22
od2 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^-)$	p23
od2 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^+)$	p24
od2 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^-)$	p25
od2 : $Pr(T3^-/ D^- \cap T1^- \cap T2^-)$	p26
od2 : $Pr(T3^-/ D^- \cap T1^- \cap T2^+)$	p27
od2 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^-)$	p28
od2 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^+)$	p29
od3 : $Pr(T1^+/D^+)$	p30
od3 : $Pr(T1^-/D^-)$	p31
od3 : $Pr(T2^+/D^+ \cap T1^+)$	p32
od3 : $Pr(T2^+/ D^+ \cap T1^-)$	p33
od3 : $Pr(T2^-/ D^- \cap T1^-)$	p34
od3 : $Pr(T2^-/ D^- \cap T1^+)$	p35
od3 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^+)$	p36
od3 : $Pr(T3^+/ D^+ \cap T1^+ \cap T2^-)$	p37
od3 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^+)$	p38
od3 : $Pr(T3^+/ D^+ \cap T1^- \cap T2^-)$	p39
od3 : $Pr(T3^-/ D^- \cap T1^- \cap T2^-)$	p40
od3 : $Pr(T3^-/ D^- \cap T1^- \cap T2^+)$	p41
od3 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^-)$	p42
od3 : $Pr(T3^-/ D^- \cap T1^+ \cap T2^+)$	p43

The probabilities of possible results with three tests at 3 possible cut-off level are given as follows:

od1: P111 =p1*p2*p4*p8+(1-p1)*(1-p3)*(1-p7)*(1-p15)
od1: P110 = p1*p2*p4*(1-p8)+(1-p1)*(1-p3)*(1-p7)*p15
od1: P101 = p1*p2*(1-p4)*p9+(1-p1)*(1-p3)*p7*(1-p14)
od1: P100 = p1*p2*(1-p4)*(1-p9)+(1-p1)*(1-p3)*p7*p14
od1: P011 = p1*(1-p2)*p5*p10+(1-p1)*p3*(1-p6)*(1-p13)
od1: P010 = p1*(1-p2)*p5*(1-p10)+(1-p1)*p3*(1-p6)*p13
od1: P001 = p1*(1-p2)*(1-p5)*p11+(1-p1)*p3*p6*(1-p12)
od1: P000 = p1*(1-p2)*(1-p5)*(1-p11)+(1-p1)*p3*p6*p12
od2: P111 = p1*p16*p18*p22+(1-p1)*(1-p17)*(1-p21)*(1-p29)
od2: P110 = p1*p16*p18*(1-p22)+(1-p1)*(1-p17)*(1-p21)*p29
od2: P101 = p1*p16*(1-p18)*p23+(1-p1)*(1-p17)*p21*(1-p28)
od2: P100 = p1*p16*(1-p18)*(1-p23)+(1-p1)*(1-p17)*p21*p28
od2: P011 = p1*(1-p16)*p19*p24+(1-p1)*p17*(1-p20)*(1-p27)
od2: P010 = p1*(1-p16)*p19*(1-p24)+(1-p1)*p17*(1-p20)*p27
od2: P001 = p1*(1-p16)*(1-p19)*p25+(1-p1)*p17*p20*(1-p26)
od2: P000 = p1*(1-p16)*(1-p19)*(1-p25)+(1-p1)*p17*p20*p26
od3: P111 = p1*p30*p32*p36+(1-p1)*(1-p31)*(1-p35)*(1-p43)
od3: P110 = p1*p30*p32*(1-p36)+(1-p1)*(1-p31)*(1-p35)*p43
od3: P101 = p1*p30*(1-p32)*p37+(1-p1)*(1-p31)*p35*(1-p42)
od3: P100 = p1*p30*(1-p32)*(1-p37)+(1-p1)*(1-p31)*p35*p42
od3: P011 = p1*(1-p30)*p33*p38+(1-p1)*p31*(1-p34)*(1-p41)
od3: P010 = p1*(1-p30)*p33*(1-p38)+(1-p1)*p31*(1-p34)*p41
od3: P001 = p1*(1-p30)*(1-p33)*p39+(1-p1)*p31*p34*(1-p40)
od3: P000 = p1*(1-p30)*(1-p33)*(1-p39)+(1-p1)*p31*p34*p40
Sensitivities and the specificities of the three tests at 3 cut-off:

Sensitivities: se1, se2 and se3 for Ag-ELISA, Ab-ELISA-CF and Ab-ELISA-F3, respectively
od1: se1= p2
od2: se1= p16
od3: se1= p30
od1: se2 = p2*p4+(1-p2)*p5
od2: se2 = p16*p18+(1-p16)*p19
od3: se2 = p30*p32+(1-p30)*p33
od1: se3 = p2*(p4*p8+(1-p4)*p9)+(1-p2)*(p5*p10+(1-p5)*p11)
od2: se3 = p16*(p18*p22+(1-p18)*p23)+(1-p16)*(p19*p24+(1-p19)*p25)
od3: se3 = p30*(p32*p36+(1-p32)*p37)+(1-p30)*(p33*p38+(1-p33)*p39).

Specificity: sp1, sp2 and sp3 for Ag-ELISA, Ab-ELISA-CF and Ab-ELISA-F3, respectively
od1: sp1 = p3
od2: sp1 = p17
od3: sp1 = p31
od1: sp2 = p3*p6+(1-p3)*p7
od2: sp2 = p17*p20+(1-p17)*p21
od3: sp2 = p31*p34+(1-p31)*p35
od1: sp3 = p3*(p6*p12+(1-p6)*p13)+(1-p3)*(p7*p14+(1-p7)*p15)
od2: sp3 = p17*(p20*p26+(1-p20)*p27)+(1-p17)*(p21*p28+(1-p21)*p29)
od3: sp3 = p31*(p34*p40+(1-p34)*p41)+(1-p31)*(p35*p42+(1-p35)*p43)

- In the case of **n** optical densities to be selected as cut-off, **n** possible sensitivities and **n** possible specificities could be estimated for each test. The true prevalence of the disease

remains constant. The number of parameters (probabilities) to be estimated is **N= 14*n+1 (Appendix 3.1)**. This means that there are 14 parameters with variable values following the changes in cut-off (optical density) while the true prevalence of disease remains constant.
The number of probabilities (Np) of possible results with three tests at **n** possible cut-off values are given as follows: **Np= 8*n**.
WinBUGS code for ROC analysis using conditional model over a range of 48 different cut-off (optical densities) is given in **Appendix 3.2**. Expert opinion was based on the calculation of the value of each parameter using previous results of the 3 tests applied on 66 serum samples from Zambia. The model was also run without expert opinion.

Appendix. 3.1- Number of parameters based on 48 different optical densities (cut-off) obtained by ELISA-F3 for detection of cysticercosis in 66 dissected Zambian pigs

Optical density (od)	Parameters														
od1: 0.16	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	p11	p12	p13	p14	p15
od2: 0.17	p1	p16	p17	p18	p19	p20	p21	p22	p23	p24	p25	p26	p27	p28	p29
od3: 0.19	p1	p30	p31	p32	p33	p34	p35	p36	p37	p38	p39	p40	p41	p42	p43
od4: 0.20	p1	p44	p45	p46	p47	p48	p49	p50	p51	p52	p53	p54	p55	p56	p57
od5: 0.22	p1	p58	p59	p60	p61	p62	p63	p64	p65	p66	p67	p68	p69	p70	p71
od6: 0.25	p1	p72	p73	p74	p75	p76	p77	p78	p79	p80	p81	p82	p83	p84	p85
od7: 0.26	p1	p86	p87	p88	p89	p90	p91	p92	p93	p94	p95	p96	p97	p98	p99
od8: 0.28	p1	p100	p101	p102	p103	p104	p105	p106	p107	p108	p109	p110	p111	p112	p113
od9: 0.29	p1	p114	p115	p116	p117	p118	p119	p120	p121	p122	p123	p124	p125	p126	p127
od10: 0.31	p1	p128	p129	p130	p131	p132	p133	p134	p135	p136	p137	p138	p139	p140	p141
od11: 0.32	p1	p142	p143	p144	p145	p146	p147	p148	p149	p150	p151	p152	p153	p154	p155
od12: 0.37	p1	p156	p157	p158	p159	p160	p161	p162	p163	p164	p165	p166	p167	p168	p169
od13: 0.38	p1	p170	p171	p172	p173	p174	p175	p176	p177	p178	p179	p180	p181	p182	p183
od14: 0.43	p1	p184	p185	p186	p187	p188	p189	p190	p191	p192	p193	p194	p195	p196	p197
od15: 0.44	p1	p198	p199	p200	p201	p202	p203	p204	p205	p206	p207	p208	p209	p210	p211
od16: 0.46	p1	p212	p213	p214	p215	p216	p217	p218	p219	p220	p221	p222	p223	p224	p225
od17: 0.47	p1	p226	p227	p228	p229	p230	p231	p232	p233	p234	p235	p236	p237	p238	p239
od18: 0.52	p1	p240	p241	p242	p243	p244	p245	p246	p247	p248	p249	p250	p251	p252	p253
od19: 0.53	p1	p254	p255	p256	p257	p258	p259	p260	p261	p262	p263	p264	p265	p266	p267
od20: 0.57	p1	p268	p269	p270	p271	p272	p273	p274	p275	p276	p277	p278	p279	p280	p281
od21: 0.59	p1	p282	p283	p284	p285	p286	p287	p288	p289	p290	p291	p292	p293	p294	p295
od22: 0.60	p1	p296	p297	p298	p299	p300	p301	p302	p303	p304	p305	p306	p307	p308	p309
od23: 0.61	p1	p310	p311	p312	p313	p314	p315	p316	p317	p318	p319	p320	p321	p322	p323
od24: 0.63	p1	p324	p325	p326	p327	p328	p329	p330	p331	p332	p333	p334	p335	p336	p337
od25: 0.65	p1	p338	p339	p340	p341	p342	p343	p344	p345	p346	p347	p348	p349	p350	p351
od26: 0.68	p1	p352	p353	p354	p355	p356	p357	p358	p359	p360	p361	p362	p363	p364	p365
od27: 0.69	p1	p366	p367	p368	p369	p370	p371	p372	p373	p374	p375	p376	p377	p378	p379
od28: 0.71	p1	p380	p381	p382	p383	p384	p385	p386	p387	p388	p389	p390	p391	p392	p393
od29: 0.73	p1	p394	p395	p396	p397	p398	p399	p400	p401	p402	p403	p404	p405	p406	p407
od30: 0.74	p1	p408	p409	p410	p411	p412	p413	p414	p415	p416	p417	p418	p419	p420	p421
od31: 0.75	p1	p422	p423	p424	p425	p426	p427	p428	p429	p430	p431	p432	p433	p434	p435
od32: 0.78	p1	p436	p437	p438	p439	p440	p441	p442	p443	p444	p445	p446	p447	p448	p449
od33: 0.79	p1	p450	p451	p452	p453	p454	p455	p456	p457	p458	p459	p460	p461	p462	p463
od34: 0.80	p1	p464	p465	p466	p467	p468	p469	p470	p471	p472	p473	p474	p475	p476	p477
od35: 0.82	p1	p478	p479	p480	p481	p482	p483	p484	p485	p486	p487	p488	p489	p490	p491
od36: 0.87	p1	p492	p493	p494	p495	p496	p497	p498	p499	p500	p501	p502	p503	p504	p505
od37: 0.89	p1	p506	p507	p508	p509	p510	p511	p512	p513	p514	p515	p516	p517	p518	p519
od38: 0.90	p1	p520	p521	p522	p523	p524	p525	p526	p527	p528	p529	p530	p531	p532	p533
od39: 0.92	p1	p534	p535	p536	p537	p538	p539	p540	p541	p542	p543	p544	p545	p546	p547
od40: 1.02	p1	p548	p549	p550	p551	p552	p553	p554	p555	p556	p557	p558	p559	p560	p561
od41: 1.03	p1	p562	p563	p564	p565	p566	p567	p568	p569	p570	p571	p572	p573	p574	p575
od42: 1.04	p1	p576	p577	p578	p579	p580	p581	p582	p583	p584	p585	p586	p587	p588	p589
od43: 1:14	p1	p590	p591	p592	p593	p594	p595	p596	p597	p598	p599	p600	p601	p602	p603
od44: 1.18	p1	p604	p605	p606	p607	p608	p609	p610	p611	p612	p613	p614	p615	p616	p617
od45: 1.47	p1	p618	p619	p620	p621	p622	p623	p624	p625	p626	p627	p628	p629	p630	p631
od46: 1.64	p1	p632	p633	p634	p635	p636	p637	p638	p639	p640	p641	p642	p643	p644	p645
od47: 1.75	p1	p646	p647	p648	p649	p650	p651	p652	p653	p654	p655	p656	p657	p658	p659
od48: 1.76	p1	p660	p661	p662	p663	p664	p665	p666	p667	p668	p669	p670	p671	p672	p673

Appendix 3.2. WinBUGS code for ROC model based on all Ab-ELISA-F3 optical densities (od) of serum samples of 66 dissected Zambian pigs.

```
model
{
rod1[1:8] ~ dmulti(prod1[1:8], n)
rod2[1:8] ~ dmulti(prod2[1:8], n)
rod3[1:8] ~ dmulti(prod3[1:8], n)
rod4[1:8] ~ dmulti(prod4[1:8], n)
rod5[1:8] ~ dmulti(prod5[1:8], n)
rod6[1:8] ~ dmulti(prod6[1:8], n)
rod7[1:8] ~ dmulti(prod7[1:8], n)
rod8[1:8] ~ dmulti(prod8[1:8], n)
rod9[1:8] ~ dmulti(prod9[1:8], n)
rod10[1:8] ~ dmulti(prod10[1:8], n)
rod11[1:8] ~ dmulti(prod11[1:8], n)
rod12[1:8] ~ dmulti(prod12[1:8], n)
rod13[1:8] ~ dmulti(prod13[1:8], n)
rod14[1:8] ~ dmulti(prod14[1:8], n)
rod15[1:8] ~ dmulti(prod15[1:8], n)
rod16[1:8] ~ dmulti(prod16[1:8], n)
rod17[1:8] ~ dmulti(prod17[1:8], n)
rod18[1:8] ~ dmulti(prod18[1:8], n)
rod19[1:8] ~ dmulti(prod19[1:8], n)
rod20[1:8] ~ dmulti(prod20[1:8], n)
rod21[1:8] ~ dmulti(prod21[1:8], n)
rod22[1:8] ~ dmulti(prod22[1:8], n)
rod23[1:8] ~ dmulti(prod23[1:8], n)
rod24[1:8] ~ dmulti(prod24[1:8], n)
rod25[1:8] ~ dmulti(prod25[1:8], n)
rod26[1:8] ~ dmulti(prod26[1:8], n)
rod27[1:8] ~ dmulti(prod27[1:8], n)
rod28[1:8] ~ dmulti(prod28[1:8], n)
rod29[1:8] ~ dmulti(prod29[1:8], n)
rod30[1:8] ~ dmulti(prod30[1:8], n)
rod31[1:8] ~ dmulti(prod31[1:8], n)
rod32[1:8] ~ dmulti(prod32[1:8], n)
rod33[1:8] ~ dmulti(prod33[1:8], n)
rod34[1:8] ~ dmulti(prod34[1:8], n)
rod35[1:8] ~ dmulti(prod35[1:8], n)
rod36[1:8] ~ dmulti(prod36[1:8], n)
rod37[1:8] ~ dmulti(prod37[1:8], n)
rod38[1:8] ~ dmulti(prod38[1:8], n)
rod39[1:8] ~ dmulti(prod39[1:8], n)
rod40[1:8] ~ dmulti(prod40[1:8], n)
rod41[1:8] ~ dmulti(prod41[1:8], n)
rod42[1:8] ~ dmulti(prod42[1:8], n)
rod43[1:8] ~ dmulti(prod43[1:8], n)
rod44[1:8] ~ dmulti(prod44[1:8], n)
rod45[1:8] ~ dmulti(prod45[1:8], n)
rod46[1:8] ~ dmulti(prod46[1:8], n)
rod47[1:8] ~ dmulti(prod47[1:8], n)
rod48[1:8] ~ dmulti(prod48[1:8], n)
        prod1[1] <-  p1*p2*p4*p8+(1-p1)*(1-p3)*(1-p7)*(1-p15)
        prod1[2] <- p1*p2*p4*(1-p8)+(1-p1)*(1-p3)*(1-p7)*p15
        prod1[3] <- p1*p2*(1-p4)*p9+(1-p1)*(1-p3)*p7*(1-p14)
        prod1[4] <- p1*p2*(1-p4)*(1-p9)+(1-p1)*(1-p3)*p7*p14
        prod1[5] <- p1*(1-p2)*p5*p10+(1-p1)*p3*(1-p6)*(1-p13)
        prod1[6] <- p1*(1-p2)*p5*(1-p10)+(1-p1)*p3*(1-p6)*p13
        prod1[7] <- p1*(1-p2)*(1-p5)*p11+(1-p1)*p3*p6*(1-p12)
        prod1[8] <- p1*(1-p2)*(1-p5)*(1-p11)+(1-p1)*p3*p6*p12
```

```
prod2[1] <-  p1*p16*p18*p22+(1-p1)*(1-p17)*(1-p21)*(1-p29)
prod2[2] <- p1*p16*p18*(1-p22)+(1-p1)*(1-p17)*(1-p21)*p29
prod2[3] <- p1*p16*(1-p18)*p23+(1-p1)*(1-p17)*p21*(1-p28)
prod2[4] <- p1*p16*(1-p18)*(1-p23)+(1-p1)*(1-p17)*p21*p28
prod2[5] <- p1*(1-p16)*p19*p24+(1-p1)*p17*(1-p20)*(1-p27)
prod2[6] <- p1*(1-p16)*p19*(1-p24)+(1-p1)*p17*(1-p20)*p27
prod2[7] <- p1*(1-p16)*(1-p19)*p25+(1-p1)*p17*p20*(1-p26)
prod2[8] <- p1*(1-p16)*(1-p19)*(1-p25)+(1-p1)*p17*p20*p26
prod3[1] <- p1*p30*p32*p36+(1-p1)*(1-p31)*(1-p35)*(1-p43)
prod3[2] <- p1*p30*p32*(1-p36)+(1-p1)*(1-p31)*(1-p35)*p43
prod3[3] <- p1*p30*(1-p32)*p37+(1-p1)*(1-p31)*p35*(1-p42)
prod3[4] <- p1*p30*(1-p32)*(1-p37)+(1-p1)*(1-p31)*p35*p42
prod3[5] <- p1*(1-p30)*p33*p38+(1-p1)*p31*(1-p34)*(1-p41)
prod3[6] <- p1*(1-p30)*p33*(1-p38)+(1-p1)*p31*(1-p34)*p41
prod3[7] <- p1*(1-p30)*(1-p33)*p39+(1-p1)*p31*p34*(1-p40)
prod3[8] <- p1*(1-p30)*(1-p33)*(1-p39)+(1-p1)*p31*p34*p40
prod4[1] <-  p1*p44*p46*p50+(1-p1)*(1-p45)*(1-p49)*(1-p57)
prod4[2] <- p1*p44*p46*(1-p50)+(1-p1)*(1-p45)*(1-p49)*p57
prod4[3] <- p1*p44*(1-p46)*p51+(1-p1)*(1-p45)*p49*(1-p56)
prod4[4] <- p1*p44*(1-p46)*(1-p51)+(1-p1)*(1-p45)*p49*p56
prod4[5] <- p1*(1-p44)*p47*p52+(1-p1)*p45*(1-p48)*(1-p55)
prod4[6] <- p1*(1-p44)*p47*(1-p52)+(1-p1)*p45*(1-p48)*p55
prod4[7] <- p1*(1-p44)*(1-p47)*p53+(1-p1)*p45*p48*(1-p54)
prod4[8] <- p1*(1-p44)*(1-p47)*(1-p53)+(1-p1)*p45*p48*p54
prod5[1] <-  p1*p58*p60*p64+(1-p1)*(1-p59)*(1-p63)*(1-p71)
prod5[2] <- p1*p58*p60*(1-p64)+(1-p1)*(1-p59)*(1-p63)*p71
prod5[3] <- p1*p58*(1-p60)*p65+(1-p1)*(1-p59)*p63*(1-p70)
prod5[4] <- p1*p58*(1-p60)*(1-p65)+(1-p1)*(1-p59)*p63*p70
prod5[5] <- p1*(1-p58)*p61*p66+(1-p1)*p59*(1-p62)*(1-p69)
prod5[6] <- p1*(1-p58)*p61*(1-p66)+(1-p1)*p59*(1-p62)*p69
prod5[7] <- p1*(1-p58)*(1-p61)*p67+(1-p1)*p59*p62*(1-p68)
prod5[8] <- p1*(1-p58)*(1-p61)*(1-p67)+(1-p1)*p59*p62*p68
prod6[1] <-  p1*p72*p74*p78+(1-p1)*(1-p73)*(1-p77)*(1-p85)
prod6[2] <- p1*p72*p74*(1-p78)+(1-p1)*(1-p73)*(1-p77)*p85
prod6[3] <- p1*p72*(1-p74)*p79+(1-p1)*(1-p73)*p77*(1-p84)
prod6[4] <- p1*p72*(1-p74)*(1-p79)+(1-p1)*(1-p73)*p77*p84
prod6[5] <- p1*(1-p72)*p75*p80+(1-p1)*p73*(1-p76)*(1-p83)
prod6[6] <- p1*(1-p72)*p75*(1-p80)+(1-p1)*p73*(1-p76)*p83
prod6[7] <- p1*(1-p72)*(1-p75)*p81+(1-p1)*p73*p76*(1-p82)
prod6[8] <- p1*(1-p72)*(1-p75)*(1-p81)+(1-p1)*p73*p76*p82
prod7[1] <-  p1*p86*p88*p92+(1-p1)*(1-p87)*(1-p91)*(1-p99)
prod7[2] <- p1*p86*p88*(1-p92)+(1-p1)*(1-p87)*(1-p91)*p99
prod7[3] <- p1*p86*(1-p88)*p93+(1-p1)*(1-p87)*p91*(1-p98)
prod7[4] <- p1*p86*(1-p88)*(1-p93)+(1-p1)*(1-p87)*p91*p98
prod7[5] <- p1*(1-p86)*p89*p94+(1-p1)*p87*(1-p90)*(1-p97)
prod7[6] <- p1*(1-p86)*p89*(1-p94)+(1-p1)*p87*(1-p90)*p97
prod7[7] <- p1*(1-p86)*(1-p89)*p95+(1-p1)*p87*p90*(1-p96)
prod7[8] <- p1*(1-p86)*(1-p89)*(1-p95)+(1-p1)*p87*p90*p96
prod8[1] <-  p1*p100*p102*p106+(1-p1)*(1-p101)*(1-p105)*(1-p113)
prod8[2] <- p1*p100*p102*(1-p106)+(1-p1)*(1-p101)*(1-p105)*p113
prod8[3] <- p1*p100*(1-p102)*p107+(1-p1)*(1-p101)*p105*(1-p112)
prod8[4] <- p1*p100*(1-p102)*(1-p107)+(1-p1)*(1-p101)*p105*p112
prod8[5] <- p1*(1-p100)*p103*p108+(1-p1)*p101*(1-p104)*(1-p111)
prod8[6] <- p1*(1-p100)*p103*(1-p108)+(1-p1)*p101*(1-p104)*p111
prod8[7] <- p1*(1-p100)*(1-p103)*p109+(1-p1)*p101*p104*(1-p110)
prod8[8] <- p1*(1-p100)*(1-p103)*(1-p109)+(1-p1)*p101*p104*p110
prod9[1] <-  p1*p114*p116*p120+(1-p1)*(1-p115)*(1-p119)*(1-p127)
prod9[2] <- p1*p114*p116*(1-p120)+(1-p1)*(1-p115)*(1-p119)*p127
prod9[3] <- p1*p114*(1-p116)*p121+(1-p1)*(1-p115)*p119*(1-p126)
prod9[4] <- p1*p114*(1-p116)*(1-p121)+(1-p1)*(1-p115)*p119*p126
```

```
prod9[5] <- p1*(1-p114)*p117*p122+(1-p1)*p115*(1-p118)*(1-p125)
prod9[6] <- p1*(1-p114)*p117*(1-p122)+(1-p1)*p115*(1-p118)*p125
prod9[7] <- p1*(1-p114)*(1-p117)*p123+(1-p1)*p115*p118*(1-p124)
prod9[8] <- p1*(1-p114)*(1-p117)*(1-p123)+(1-p1)*p115*p118*p124
prod10[1] <-  p1*p128*p130*p134+(1-p1)*(1-p129)*(1-p133)*(1-p141)
prod10[2] <- p1*p128*p130*(1-p134)+(1-p1)*(1-p129)*(1-p133)*p141
prod10[3] <- p1*p128*(1-p130)*p135+(1-p1)*(1-p129)*p133*(1-p140)
prod10[4] <- p1*p128*(1-p130)*(1-p135)+(1-p1)*(1-p129)*p133*p140
prod10[5] <- p1*(1-p128)*p131*p136+(1-p1)*p129*(1-p132)*(1-p139)
prod10[6] <- p1*(1-p128)*p131*(1-p136)+(1-p1)*p129*(1-p132)*p139
prod10[7] <- p1*(1-p128)*(1-p131)*p137+(1-p1)*p129*p132*(1-p138)
prod10[8] <- p1*(1-p128)*(1-p131)*(1-p137)+(1-p1)*p129*p132*p138
prod11[1] <-  p1*p142*p144*p148+(1-p1)*(1-p143)*(1-p147)*(1-p155)
prod11[2] <- p1*p142*p144*(1-p148)+(1-p1)*(1-p143)*(1-p147)*p155
prod11[3] <- p1*p142*(1-p144)*p149+(1-p1)*(1-p143)*p147*(1-p154)
prod11[4] <- p1*p142*(1-p144)*(1-p149)+(1-p1)*(1-p143)*p147*p154
prod11[5] <- p1*(1-p142)*p145*p150+(1-p1)*p143*(1-p146)*(1-p153)
prod11[6] <- p1*(1-p142)*p145*(1-p150)+(1-p1)*p143*(1-p146)*p153
prod11[7] <- p1*(1-p142)*(1-p145)*p151+(1-p1)*p143*p146*(1-p152)
prod11[8] <- p1*(1-p142)*(1-p145)*(1-p151)+(1-p1)*p143*p146*p152
prod12[1] <- p1*p156*p158*p162+(1-p1)*(1-p157)*(1-p161)*(1-p169)
prod12[2] <- p1*p156*p158*(1-p162)+(1-p1)*(1-p157)*(1-p161)*p169
prod12[3] <- p1*p156*(1-p158)*p163+(1-p1)*(1-p157)*p161*(1-p168)
prod12[4] <- p1*p156*(1-p158)*(1-p163)+(1-p1)*(1-p157)*p161*p168
prod12[5] <- p1*(1-p156)*p159*p164+(1-p1)*p157*(1-p160)*(1-p167)
prod12[6] <- p1*(1-p156)*p159*(1-p164)+(1-p1)*p157*(1-p160)*p167
prod12[7] <- p1*(1-p156)*(1-p159)*p165+(1-p1)*p157*p160*(1-p166)
prod12[8] <- p1*(1-p156)*(1-p159)*(1-p165)+(1-p1)*p157*p160*p166
prod13[1] <-  p1*p170*p172*p176+(1-p1)*(1-p171)*(1-p175)*(1-p183)
prod13[2] <- p1*p170*p172*(1-p176)+(1-p1)*(1-p171)*(1-p175)*p183
prod13[3] <- p1*p170*(1-p172)*p177+(1-p1)*(1-p171)*p175*(1-p182)
prod13[4] <- p1*p170*(1-p172)*(1-p177)+(1-p1)*(1-p171)*p175*p182
prod13[5] <- p1*(1-p170)*p173*p178+(1-p1)*p171*(1-p174)*(1-p181)
prod13[6] <- p1*(1-p170)*p173*(1-p178)+(1-p1)*p171*(1-p174)*p181
prod13[7] <- p1*(1-p170)*(1-p173)*p179+(1-p1)*p171*p174*(1-p180)
prod13[8] <- p1*(1-p170)*(1-p173)*(1-p179)+(1-p1)*p171*p174*p180
prod14[1] <-  p1*p184*p186*p190+(1-p1)*(1-p185)*(1-p189)*(1-p197)
prod14[2] <- p1*p184*p186*(1-p190)+(1-p1)*(1-p185)*(1-p189)*p197
prod14[3] <- p1*p184*(1-p186)*p191+(1-p1)*(1-p185)*p189*(1-p196)
prod14[4] <- p1*p184*(1-p186)*(1-p191)+(1-p1)*(1-p185)*p189*p196
prod14[5] <- p1*(1-p184)*p187*p192+(1-p1)*p185*(1-p188)*(1-p195)
prod14[6] <- p1*(1-p184)*p187*(1-p192)+(1-p1)*p185*(1-p188)*p195
prod14[7] <- p1*(1-p184)*(1-p187)*p193+(1-p1)*p185*p188*(1-p194)
prod14[8] <- p1*(1-p184)*(1-p187)*(1-p193)+(1-p1)*p185*p188*p194
prod15[1] <-  p1*p198*p200*p204+(1-p1)*(1-p199)*(1-p203)*(1-p211)
prod15[2] <- p1*p198*p200*(1-p204)+(1-p1)*(1-p199)*(1-p203)*p211
prod15[3] <- p1*p198*(1-p200)*p205+(1-p1)*(1-p199)*p203*(1-p210)
prod15[4] <- p1*p198*(1-p200)*(1-p205)+(1-p1)*(1-p199)*p203*p210
prod15[5] <- p1*(1-p198)*p201*p206+(1-p1)*p199*(1-p202)*(1-p209)
prod15[6] <- p1*(1-p198)*p201*(1-p206)+(1-p1)*p199*(1-p202)*p209
prod15[7] <- p1*(1-p198)*(1-p201)*p207+(1-p1)*p199*p202*(1-p208)
prod15[8] <- p1*(1-p198)*(1-p201)*(1-p207)+(1-p1)*p199*p202*p208
prod16[1] <- p1*p212*p214*p218+(1-p1)*(1-p213)*(1-p217)*(1-p225)
prod16[2] <- p1*p212*p214*(1-p218)+(1-p1)*(1-p213)*(1-p217)*p225
prod16[3] <- p1*p212*(1-p214)*p219+(1-p1)*(1-p213)*p217*(1-p224)
prod16[4] <- p1*p212*(1-p214)*(1-p219)+(1-p1)*(1-p213)*p217*p224
prod16[5] <- p1*(1-p212)*p215*p220+(1-p1)*p213*(1-p216)*(1-p223)
prod16[6] <- p1*(1-p212)*p215*(1-p220)+(1-p1)*p213*(1-p216)*p223
prod16[7] <- p1*(1-p212)*(1-p215)*p221+(1-p1)*p213*p216*(1-p222)
prod16[8] <- p1*(1-p212)*(1-p215)*(1-p221)+(1-p1)*p213*p216*p222
```

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prod17[1] <-  p1*p226*p228*p232+(1-p1)*(1-p227)*(1-p231)*(1-p239)
prod17[2] <- p1*p226*p228*(1-p232)+(1-p1)*(1-p227)*(1-p231)*p239
prod17[3] <- p1*p226*(1-p228)*p233+(1-p1)*(1-p227)*p231*(1-p238)
prod17[4] <- p1*p226*(1-p228)*(1-p233)+(1-p1)*(1-p227)*p231*p238
prod17[5] <- p1*(1-p226)*p229*p234+(1-p1)*p227*(1-p230)*(1-p237)
prod17[6] <- p1*(1-p226)*p229*(1-p234)+(1-p1)*p227*(1-p230)*p237
prod17[7] <- p1*(1-p226)*(1-p229)*p235+(1-p1)*p227*p230*(1-p236)
prod17[8] <- p1*(1-p226)*(1-p229)*(1-p235)+(1-p1)*p227*p230*p236
prod18[1] <-  p1*p240*p242*p246+(1-p1)*(1-p241)*(1-p245)*(1-p253)
prod18[2] <- p1*p240*p242*(1-p246)+(1-p1)*(1-p241)*(1-p245)*p253
prod18[3] <- p1*p240*(1-p242)*p247+(1-p1)*(1-p241)*p245*(1-p252)
prod18[4] <- p1*p240*(1-p242)*(1-p247)+(1-p1)*(1-p241)*p245*p252
prod18[5] <- p1*(1-p240)*p243*p248+(1-p1)*p241*(1-p244)*(1-p251)
prod18[6] <- p1*(1-p240)*p243*(1-p248)+(1-p1)*p241*(1-p244)*p251
prod18[7] <- p1*(1-p240)*(1-p243)*p249+(1-p1)*p241*p244*(1-p250)
prod18[8] <- p1*(1-p240)*(1-p243)*(1-p249)+(1-p1)*p241*p244*p250
prod19[1] <-  p1*p254*p256*p260+(1-p1)*(1-p255)*(1-p259)*(1-p267)
prod19[2] <- p1*p254*p256*(1-p260)+(1-p1)*(1-p255)*(1-p259)*p267
prod19[3] <- p1*p254*(1-p256)*p261+(1-p1)*(1-p255)*p259*(1-p266)
prod19[4] <- p1*p254*(1-p256)*(1-p261)+(1-p1)*(1-p255)*p259*p266
prod19[5] <- p1*(1-p254)*p257*p262+(1-p1)*p255*(1-p258)*(1-p265)
prod19[6] <- p1*(1-p254)*p257*(1-p262)+(1-p1)*p255*(1-p258)*p265
prod19[7] <- p1*(1-p254)*(1-p257)*p263+(1-p1)*p255*p258*(1-p264)
prod19[8] <- p1*(1-p254)*(1-p257)*(1-p263)+(1-p1)*p255*p258*p264
prod20[1] <- p1*p268*p270*p274+(1-p1)*(1-p269)*(1-p273)*(1-p281)
prod20[2] <- p1*p268*p270*(1-p274)+(1-p1)*(1-p269)*(1-p273)*p281
prod20[3] <- p1*p268*(1-p270)*p275+(1-p1)*(1-p269)*p273*(1-p280)
prod20[4] <- p1*p268*(1-p270)*(1-p275)+(1-p1)*(1-p269)*p273*p280
prod20[5] <- p1*(1-p268)*p271*p276+(1-p1)*p269*(1-p272)*(1-p279)
prod20[6] <- p1*(1-p268)*p271*(1-p276)+(1-p1)*p269*(1-p272)*p279
prod20[7] <- p1*(1-p268)*(1-p271)*p277+(1-p1)*p269*p272*(1-p278)
prod20[8] <- p1*(1-p268)*(1-p271)*(1-p277)+(1-p1)*p269*p272*p278
prod21[1] <-  p1*p282*p284*p288+(1-p1)*(1-p283)*(1-p287)*(1-p295)
prod21[2] <- p1*p282*p284*(1-p288)+(1-p1)*(1-p283)*(1-p287)*p295
prod21[3] <- p1*p282*(1-p284)*p289+(1-p1)*(1-p283)*p287*(1-p294)
prod21[4] <- p1*p282*(1-p284)*(1-p289)+(1-p1)*(1-p283)*p287*p294
prod21[5] <- p1*(1-p282)*p285*p290+(1-p1)*p283*(1-p286)*(1-p293)
prod21[6] <- p1*(1-p282)*p285*(1-p290)+(1-p1)*p283*(1-p286)*p293
prod21[7] <- p1*(1-p282)*(1-p285)*p291+(1-p1)*p283*p286*(1-p292)
prod21[8] <- p1*(1-p282)*(1-p285)*(1-p291)+(1-p1)*p283*p286*p292
prod22[1] <-  p1*p296*p298*p302+(1-p1)*(1-p297)*(1-p301)*(1-p309)
prod22[2] <- p1*p296*p298*(1-p302)+(1-p1)*(1-p297)*(1-p301)*p309
prod22[3] <- p1*p296*(1-p298)*p303+(1-p1)*(1-p297)*p301*(1-p308)
prod22[4] <- p1*p296*(1-p298)*(1-p303)+(1-p1)*(1-p297)*p301*p308
prod22[5] <- p1*(1-p296)*p299*p304+(1-p1)*p297*(1-p300)*(1-p307)
prod22[6] <- p1*(1-p296)*p299*(1-p304)+(1-p1)*p297*(1-p300)*p307
prod22[7] <- p1*(1-p296)*(1-p299)*p305+(1-p1)*p297*p300*(1-p306)
prod22[8] <- p1*(1-p296)*(1-p299)*(1-p305)+(1-p1)*p297*p300*p306
prod23[1] <-  p1*p310*p312*p316+(1-p1)*(1-p311)*(1-p315)*(1-p323)
prod23[2] <- p1*p310*p312*(1-p316)+(1-p1)*(1-p311)*(1-p315)*p323
prod23[3] <- p1*p310*(1-p312)*p317+(1-p1)*(1-p311)*p315*(1-p322)
prod23[4] <- p1*p310*(1-p312)*(1-p317)+(1-p1)*(1-p311)*p315*p322
prod23[5] <- p1*(1-p310)*p313*p318+(1-p1)*p311*(1-p314)*(1-p321)
prod23[6] <- p1*(1-p310)*p313*(1-p318)+(1-p1)*p311*(1-p314)*p321
prod23[7] <- p1*(1-p310)*(1-p313)*p319+(1-p1)*p311*p314*(1-p320)
prod23[8] <- p1*(1-p310)*(1-p313)*(1-p319)+(1-p1)*p311*p314*p320
prod24[1] <- p1*p324*p326*p330+(1-p1)*(1-p325)*(1-p329)*(1-p337)
prod24[2] <- p1*p324*p326*(1-p330)+(1-p1)*(1-p325)*(1-p329)*p337
prod24[3] <- p1*p324*(1-p326)*p331+(1-p1)*(1-p325)*p329*(1-p336)
prod24[4] <- p1*p324*(1-p326)*(1-p331)+(1-p1)*(1-p325)*p329*p336
```

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prod24[5] <- p1*(1-p324)*p327*p332+(1-p1)*p325*(1-p328)*(1-p335)
prod24[6] <- p1*(1-p324)*p327*(1-p332)+(1-p1)*p325*(1-p328)*p335
prod24[7] <- p1*(1-p324)*(1-p327)*p333+(1-p1)*p325*p328*(1-p334)
prod24[8] <- p1*(1-p324)*(1-p327)*(1-p333)+(1-p1)*p325*p328*p334
prod25[1] <-  p1*p338*p340*p344+(1-p1)*(1-p339)*(1-p343)*(1-p351)
prod25[2] <- p1*p338*p340*(1-p344)+(1-p1)*(1-p339)*(1-p343)*p351
prod25[3] <- p1*p338*(1-p340)*p345+(1-p1)*(1-p339)*p343*(1-p350)
prod25[4] <- p1*p338*(1-p340)*(1-p345)+(1-p1)*(1-p339)*p343*p350
prod25[5] <- p1*(1-p338)*p341*p346+(1-p1)*p339*(1-p342)*(1-p349)
prod25[6] <- p1*(1-p338)*p341*(1-p346)+(1-p1)*p339*(1-p342)*p349
prod25[7] <- p1*(1-p338)*(1-p341)*p347+(1-p1)*p339*p342*(1-p348)
prod25[8] <- p1*(1-p338)*(1-p341)*(1-p347)+(1-p1)*p339*p342*p348
prod26[1] <-  p1*p352*p354*p358+(1-p1)*(1-p353)*(1-p357)*(1-p365)
prod26[2] <- p1*p352*p354*(1-p358)+(1-p1)*(1-p353)*(1-p357)*p365
prod26[3] <- p1*p352*(1-p354)*p359+(1-p1)*(1-p353)*p357*(1-p364)
prod26[4] <- p1*p352*(1-p354)*(1-p359)+(1-p1)*(1-p353)*p357*p364
prod26[5] <- p1*(1-p352)*p355*p360+(1-p1)*p353*(1-p356)*(1-p363)
prod26[6] <- p1*(1-p352)*p355*(1-p360)+(1-p1)*p353*(1-p356)*p363
prod26[7] <- p1*(1-p352)*(1-p355)*p361+(1-p1)*p353*p356*(1-p362)
prod26[8] <- p1*(1-p352)*(1-p355)*(1-p361)+(1-p1)*p353*p356*p362
prod27[1] <-  p1*p366*p368*p372+(1-p1)*(1-p367)*(1-p371)*(1-p379)
prod27[2] <- p1*p366*p368*(1-p372)+(1-p1)*(1-p367)*(1-p371)*p379
prod27[3] <- p1*p366*(1-p368)*p373+(1-p1)*(1-p367)*p371*(1-p378)
prod27[4] <- p1*p366*(1-p368)*(1-p373)+(1-p1)*(1-p367)*p371*p378
prod27[5] <- p1*(1-p366)*p369*p374+(1-p1)*p367*(1-p370)*(1-p377)
prod27[6] <- p1*(1-p366)*p369*(1-p374)+(1-p1)*p367*(1-p370)*p377
prod27[7] <- p1*(1-p366)*(1-p369)*p375+(1-p1)*p367*p370*(1-p376)
prod27[8] <- p1*(1-p366)*(1-p369)*(1-p375)+(1-p1)*p367*p370*p376
prod28[1] <-  p1*p380*p382*p386+(1-p1)*(1-p381)*(1-p385)*(1-p393)
prod28[2] <- p1*p380*p382*(1-p386)+(1-p1)*(1-p381)*(1-p385)*p393
prod28[3] <- p1*p380*(1-p382)*p387+(1-p1)*(1-p381)*p385*(1-p392)
prod28[4] <- p1*p380*(1-p382)*(1-p387)+(1-p1)*(1-p381)*p385*p392
prod28[5] <- p1*(1-p380)*p383*p388+(1-p1)*p381*(1-p384)*(1-p391)
prod28[6] <- p1*(1-p380)*p383*(1-p388)+(1-p1)*p381*(1-p384)*p391
prod28[7] <- p1*(1-p380)*(1-p383)*p389+(1-p1)*p381*p384*(1-p390)
prod28[8] <- p1*(1-p380)*(1-p383)*(1-p389)+(1-p1)*p381*p384*p390
prod29[1] <-  p1*p394*p396*p400+(1-p1)*(1-p395)*(1-p399)*(1-p407)
prod29[2] <- p1*p394*p396*(1-p400)+(1-p1)*(1-p395)*(1-p399)*p407
prod29[3] <- p1*p394*(1-p396)*p401+(1-p1)*(1-p395)*p399*(1-p406)
prod29[4] <- p1*p394*(1-p396)*(1-p401)+(1-p1)*(1-p395)*p399*p406
prod29[5] <- p1*(1-p394)*p397*p402+(1-p1)*p395*(1-p398)*(1-p405)
prod29[6] <- p1*(1-p394)*p397*(1-p402)+(1-p1)*p395*(1-p398)*p405
prod29[7] <- p1*(1-p394)*(1-p397)*p403+(1-p1)*p395*p398*(1-p404)
prod29[8] <- p1*(1-p394)*(1-p397)*(1-p403)+(1-p1)*p395*p398*p404
prod30[1] <-  p1*p408*p410*p414+(1-p1)*(1-p409)*(1-p413)*(1-p421)
prod30[2] <- p1*p408*p410*(1-p414)+(1-p1)*(1-p409)*(1-p413)*p421
prod30[3] <- p1*p408*(1-p410)*p415+(1-p1)*(1-p409)*p413*(1-p420)
prod30[4] <- p1*p408*(1-p410)*(1-p415)+(1-p1)*(1-p409)*p413*p420
prod30[5] <- p1*(1-p408)*p411*p416+(1-p1)*p409*(1-p412)*(1-p419)
prod30[6] <- p1*(1-p408)*p411*(1-p416)+(1-p1)*p409*(1-p412)*p419
prod30[7] <- p1*(1-p408)*(1-p411)*p417+(1-p1)*p409*p412*(1-p418)
prod30[8] <- p1*(1-p408)*(1-p411)*(1-p417)+(1-p1)*p409*p412*p418
prod31[1] <-  p1*p422*p424*p428+(1-p1)*(1-p423)*(1-p427)*(1-p435)
prod31[2] <- p1*p422*p424*(1-p428)+(1-p1)*(1-p423)*(1-p427)*p435
prod31[3] <- p1*p422*(1-p424)*p429+(1-p1)*(1-p423)*p427*(1-p434)
prod31[4] <- p1*p422*(1-p424)*(1-p429)+(1-p1)*(1-p423)*p427*p434
prod31[5] <- p1*(1-p422)*p425*p430+(1-p1)*p423*(1-p426)*(1-p433)
prod31[6] <- p1*(1-p422)*p425*(1-p430)+(1-p1)*p423*(1-p426)*p433
prod31[7] <- p1*(1-p422)*(1-p425)*p431+(1-p1)*p423*p426*(1-p432)
prod31[8] <- p1*(1-p422)*(1-p425)*(1-p431)+(1-p1)*p423*p426*p432
```

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prod32[1] <- p1*p436*p438*p442+(1-p1)*(1-p437)*(1-p441)*(1-p449)
prod32[2] <- p1*p436*p438*(1-p442)+(1-p1)*(1-p437)*(1-p441)*p449
prod32[3] <- p1*p436*(1-p438)*p443+(1-p1)*(1-p437)*p441*(1-p448)
prod32[4] <- p1*p436*(1-p438)*(1-p443)+(1-p1)*(1-p437)*p441*p448
prod32[5] <- p1*(1-p436)*p439*p444+(1-p1)*p437*(1-p440)*(1-p447)
prod32[6] <- p1*(1-p436)*p439*(1-p444)+(1-p1)*p437*(1-p440)*p447
prod32[7] <- p1*(1-p436)*(1-p439)*p445+(1-p1)*p437*p440*(1-p446)
prod32[8] <- p1*(1-p436)*(1-p439)*(1-p445)+(1-p1)*p437*p440*p446
prod33[1] <-  p1*p450*p452*p456+(1-p1)*(1-p451)*(1-p455)*(1-p463)
prod33[2] <- p1*p450*p452*(1-p456)+(1-p1)*(1-p451)*(1-p455)*p463
prod33[3] <- p1*p450*(1-p452)*p457+(1-p1)*(1-p451)*p455*(1-p462)
prod33[4] <- p1*p450*(1-p452)*(1-p457)+(1-p1)*(1-p451)*p455*p462
prod33[5] <- p1*(1-p450)*p453*p458+(1-p1)*p451*(1-p456)*(1-p461)
prod33[6] <- p1*(1-p450)*p453*(1-p458)+(1-p1)*p451*(1-p456)*p461
prod33[7] <- p1*(1-p450)*(1-p453)*p459+(1-p1)*p451*p456*(1-p460)
prod33[8] <- p1*(1-p450)*(1-p453)*(1-p459)+(1-p1)*p451*p456*p460
prod34[1] <-  p1*p464*p466*p470+(1-p1)*(1-p465)*(1-p469)*(1-p477)
prod34[2] <- p1*p464*p466*(1-p470)+(1-p1)*(1-p465)*(1-p469)*p477
prod34[3] <- p1*p464*(1-p466)*p471+(1-p1)*(1-p465)*p469*(1-p476)
prod34[4] <- p1*p464*(1-p466)*(1-p471)+(1-p1)*(1-p465)*p469*p476
prod34[5] <- p1*(1-p464)*p467*p472+(1-p1)*p465*(1-p468)*(1-p475)
prod34[6] <- p1*(1-p464)*p467*(1-p472)+(1-p1)*p465*(1-p468)*p475
prod34[7] <- p1*(1-p464)*(1-p467)*p473+(1-p1)*p465*p468*(1-p474)
prod34[8] <- p1*(1-p464)*(1-p467)*(1-p473)+(1-p1)*p465*p468*p474
prod35[1] <-  p1*p478*p480*p484+(1-p1)*(1-p479)*(1-p483)*(1-p491)
prod35[2] <- p1*p478*p480*(1-p484)+(1-p1)*(1-p479)*(1-p483)*p491
prod35[3] <- p1*p478*(1-p480)*p485+(1-p1)*(1-p479)*p483*(1-p490)
prod35[4] <- p1*p478*(1-p480)*(1-p485)+(1-p1)*(1-p479)*p483*p490
prod35[5] <- p1*(1-p478)*p481*p486+(1-p1)*p479*(1-p482)*(1-p489)
prod35[6] <- p1*(1-p478)*p481*(1-p486)+(1-p1)*p479*(1-p482)*p489
prod35[7] <- p1*(1-p478)*(1-p481)*p487+(1-p1)*p479*p482*(1-p488)
prod35[8] <- p1*(1-p478)*(1-p481)*(1-p487)+(1-p1)*p479*p482*p488
prod36[1] <-  p1*p492*p494*p498+(1-p1)*(1-p492)*(1-p497)*(1-p505)
prod36[2] <- p1*p492*p494*(1-p498)+(1-p1)*(1-p492)*(1-p497)*p505
prod36[3] <- p1*p492*(1-p494)*p499+(1-p1)*(1-p492)*p497*(1-p504)
prod36[4] <- p1*p492*(1-p494)*(1-p499)+(1-p1)*(1-p492)*p497*p504
prod36[5] <- p1*(1-p492)*p495*p500+(1-p1)*p492*(1-p496)*(1-p503)
prod36[6] <- p1*(1-p492)*p495*(1-p500)+(1-p1)*p492*(1-p496)*p503
prod36[7] <- p1*(1-p492)*(1-p495)*p501+(1-p1)*p492*p496*(1-p502)
prod36[8] <- p1*(1-p492)*(1-p495)*(1-p501)+(1-p1)*p492*p496*p502
prod37[1] <-  p1*p506*p508*p512+(1-p1)*(1-p507)*(1-p511)*(1-p519)
prod37[2] <- p1*p506*p508*(1-p512)+(1-p1)*(1-p507)*(1-p511)*p519
prod37[3] <- p1*p506*(1-p508)*p513+(1-p1)*(1-p507)*p511*(1-p518)
prod37[4] <- p1*p506*(1-p508)*(1-p513)+(1-p1)*(1-p507)*p511*p518
prod37[5] <- p1*(1-p506)*p509*p514+(1-p1)*p507*(1-p510)*(1-p517)
prod37[6] <- p1*(1-p506)*p509*(1-p514)+(1-p1)*p507*(1-p510)*p517
prod37[7] <- p1*(1-p506)*(1-p509)*p515+(1-p1)*p507*p510*(1-p516)
prod37[8] <- p1*(1-p506)*(1-p509)*(1-p515)+(1-p1)*p507*p510*p516
prod38[1] <-  p1*p520*p522*p526+(1-p1)*(1-p521)*(1-p525)*(1-p533)
prod38[2] <- p1*p520*p522*(1-p526)+(1-p1)*(1-p521)*(1-p525)*p533
prod38[3] <- p1*p520*(1-p522)*p527+(1-p1)*(1-p521)*p525*(1-p532)
prod38[4] <- p1*p520*(1-p522)*(1-p527)+(1-p1)*(1-p521)*p525*p532
prod38[5] <- p1*(1-p520)*p523*p528+(1-p1)*p521*(1-p524)*(1-p531)
prod38[6] <- p1*(1-p520)*p523*(1-p528)+(1-p1)*p521*(1-p524)*p531
prod38[7] <- p1*(1-p520)*(1-p523)*p529+(1-p1)*p521*p524*(1-p530)
prod38[8] <- p1*(1-p520)*(1-p523)*(1-p529)+(1-p1)*p521*p524*p530
prod39[1] <-  p1*p534*p536*p540+(1-p1)*(1-p535)*(1-p539)*(1-p547)
prod39[2] <- p1*p534*p536*(1-p540)+(1-p1)*(1-p535)*(1-p539)*p547
prod39[3] <- p1*p534*(1-p536)*p541+(1-p1)*(1-p535)*p539*(1-p546)
prod39[4] <- p1*p534*(1-p536)*(1-p541)+(1-p1)*(1-p535)*p539*p546
```

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prod39[5] <- p1*(1-p534)*p537*p542+(1-p1)*p535*(1-p538)*(1-p545)
prod39[6] <- p1*(1-p534)*p537*(1-p542)+(1-p1)*p535*(1-p538)*p545
prod39[7] <- p1*(1-p534)*(1-p537)*p543+(1-p1)*p535*p538*(1-p544)
prod39[8] <- p1*(1-p534)*(1-p537)*(1-p543)+(1-p1)*p535*p538*p544
prod40[1] <-  p1*p548*p550*p554+(1-p1)*(1-p549)*(1-p553)*(1-p561)
prod40[2] <- p1*p548*p550*(1-p554)+(1-p1)*(1-p549)*(1-p553)*p561
prod40[3] <- p1*p548*(1-p550)*p555+(1-p1)*(1-p549)*p553*(1-p560)
prod40[4] <- p1*p548*(1-p550)*(1-p555)+(1-p1)*(1-p549)*p553*p560
prod40[5] <- p1*(1-p548)*p551*p556+(1-p1)*p549*(1-p552)*(1-p559)
prod40[6] <- p1*(1-p548)*p551*(1-p556)+(1-p1)*p549*(1-p552)*p559
prod40[7] <- p1*(1-p548)*(1-p551)*p557+(1-p1)*p549*p552*(1-p558)
prod40[8] <- p1*(1-p548)*(1-p551)*(1-p557)+(1-p1)*p549*p552*p558
prod41[1] <- p1*p562*p564*p568+(1-p1)*(1-p563)*(1-p567)*(1-p575)
prod41[2] <- p1*p562*p564*(1-p568)+(1-p1)*(1-p563)*(1-p567)*p575
prod41[3] <- p1*p562*(1-p564)*p569+(1-p1)*(1-p563)*p567*(1-p574)
prod41[4] <- p1*p562*(1-p564)*(1-p569)+(1-p1)*(1-p563)*p567*p574
prod41[5] <- p1*(1-p562)*p565*p570+(1-p1)*p563*(1-p566)*(1-p573)
prod41[6] <- p1*(1-p562)*p565*(1-p570)+(1-p1)*p563*(1-p566)*p573
prod41[7] <- p1*(1-p562)*(1-p565)*p571+(1-p1)*p563*p566*(1-p572)
prod41[8] <- p1*(1-p562)*(1-p565)*(1-p571)+(1-p1)*p563*p566*p572
prod42[1] <- p1*p576*p578*p582+(1-p1)*(1-p577)*(1-p581)*(1-p589)
prod42[2] <- p1*p576*p578*(1-p582)+(1-p1)*(1-p577)*(1-p581)*p589
prod42[3] <- p1*p576*(1-p578)*p583+(1-p1)*(1-p577)*p581*(1-p588)
prod42[4] <- p1*p576*(1-p578)*(1-p583)+(1-p1)*(1-p577)*p581*p588
prod42[5] <- p1*(1-p576)*p579*p584+(1-p1)*p577*(1-p580)*(1-p587)
prod42[6] <- p1*(1-p576)*p579*(1-p584)+(1-p1)*p577*(1-p580)*p587
prod42[7] <- p1*(1-p576)*(1-p579)*p585+(1-p1)*p577*p580*(1-p586)
prod42[8] <- p1*(1-p576)*(1-p579)*(1-p585)+(1-p1)*p577*p580*p586
prod43[1] <-  p1*p590*p592*p596+(1-p1)*(1-p591)*(1-p595)*(1-p603)
prod43[2] <- p1*p590*p592*(1-p596)+(1-p1)*(1-p591)*(1-p595)*p603
prod43[3] <- p1*p590*(1-p592)*p597+(1-p1)*(1-p591)*p595*(1-p602)
prod43[4] <- p1*p590*(1-p592)*(1-p597)+(1-p1)*(1-p591)*p595*p602
prod43[5] <- p1*(1-p590)*p593*p598+(1-p1)*p591*(1-p594)*(1-p601)
prod43[6] <- p1*(1-p590)*p593*(1-p598)+(1-p1)*p591*(1-p594)*p601
prod43[7] <- p1*(1-p590)*(1-p593)*p599+(1-p1)*p591*p594*(1-p600)
prod43[8] <- p1*(1-p590)*(1-p593)*(1-p599)+(1-p1)*p591*p594*p600
prod44[1] <-  p1*p604*p606*p610+(1-p1)*(1-p605)*(1-p609)*(1-p617)
prod44[2] <- p1*p604*p606*(1-p610)+(1-p1)*(1-p605)*(1-p609)*p617
prod44[3] <- p1*p604*(1-p606)*p611+(1-p1)*(1-p605)*p609*(1-p616)
prod44[4] <- p1*p604*(1-p606)*(1-p611)+(1-p1)*(1-p605)*p609*p616
prod44[5] <- p1*(1-p604)*p607*p612+(1-p1)*p605*(1-p608)*(1-p615)
prod44[6] <- p1*(1-p604)*p607*(1-p612)+(1-p1)*p605*(1-p608)*p615
prod44[7] <- p1*(1-p604)*(1-p607)*p613+(1-p1)*p605*p608*(1-p614)
prod44[8] <- p1*(1-p604)*(1-p607)*(1-p613)+(1-p1)*p605*p608*p614
prod45[1] <-  p1*p618*p620*p624+(1-p1)*(1-p619)*(1-p623)*(1-p631)
prod45[2] <- p1*p618*p620*(1-p624)+(1-p1)*(1-p619)*(1-p623)*p631
prod45[3] <- p1*p618*(1-p620)*p625+(1-p1)*(1-p619)*p623*(1-p630)
prod45[4] <- p1*p618*(1-p620)*(1-p625)+(1-p1)*(1-p619)*p623*p630
prod45[5] <- p1*(1-p618)*p621*p626+(1-p1)*p619*(1-p622)*(1-p629)
prod45[6] <- p1*(1-p618)*p621*(1-p626)+(1-p1)*p619*(1-p622)*p629
prod45[7] <- p1*(1-p618)*(1-p621)*p627+(1-p1)*p619*p622*(1-p628)
prod45[8] <- p1*(1-p618)*(1-p621)*(1-p627)+(1-p1)*p619*p622*p628
prod46[1] <-  p1*p632*p634*p638+(1-p1)*(1-p633)*(1-p637)*(1-p645)
prod46[2] <- p1*p632*p634*(1-p638)+(1-p1)*(1-p633)*(1-p637)*p645
prod46[3] <- p1*p632*(1-p634)*p639+(1-p1)*(1-p633)*p637*(1-p644)
prod46[4] <- p1*p632*(1-p634)*(1-p639)+(1-p1)*(1-p633)*p637*p644
prod46[5] <- p1*(1-p632)*p635*p640+(1-p1)*p633*(1-p636)*(1-p643)
prod46[6] <- p1*(1-p632)*p635*(1-p640)+(1-p1)*p633*(1-p636)*p643
prod46[7] <- p1*(1-p632)*(1-p635)*p641+(1-p1)*p633*p636*(1-p642)
prod46[8] <- p1*(1-p632)*(1-p635)*(1-p641)+(1-p1)*p633*p636*p642
```

prod47[1] <- p1*p646*p648*p652+(1-p1)*(1-p647)*(1-p651)*(1-p659)
prod47[2] <- p1*p646*p648*(1-p652)+(1-p1)*(1-p647)*(1-p651)*p659
prod47[3] <- p1*p646*(1-p648)*p653+(1-p1)*(1-p647)*p651*(1-p658)
prod47[4] <- p1*p646*(1-p648)*(1-p653)+(1-p1)*(1-p647)*p651*p658
prod47[5] <- p1*(1-p646)*p649*p654+(1-p1)*p647*(1-p650)*(1-p657)
prod47[6] <- p1*(1-p646)*p649*(1-p654)+(1-p1)*p647*(1-p650)*p657
prod47[7] <- p1*(1-p646)*(1-p649)*p655+(1-p1)*p647*p650*(1-p656)
prod47[8] <- p1*(1-p646)*(1-p649)*(1-p655)+(1-p1)*p647*p650*p656
prod48[1] <- p1*p660*p662*p666+(1-p1)*(1-p661)*(1-p665)*(1-p673)
prod48[2] <- p1*p660*p662*(1-p666)+(1-p1)*(1-p661)*(1-p665)*p673
prod48[3] <- p1*p660*(1-p662)*p667+(1-p1)*(1-p661)*p665*(1-p672)
prod48[4] <- p1*p660*(1-p662)*(1-p667)+(1-p1)*(1-p661)*p665*p672
prod48[5] <- p1*(1-p660)*p663*p668+(1-p1)*p661*(1-p664)*(1-p671)
prod48[6] <- p1*(1-p660)*p663*(1-p668)+(1-p1)*p661*(1-p664)*p671
prod48[7] <- p1*(1-p660)*(1-p663)*p669+(1-p1)*p661*p664*(1-p670)
prod48[8] <- p1*(1-p660)*(1-p663)*(1-p669)+(1-p1)*p661*p664*p670

p1 ~ dbeta(1,1)
p2 ~ dbeta(1,1)
p3 ~ dbeta(1,1)
p4 ~ dbeta(1,1)
p5 ~ dbeta(1,1)
p6 ~ dbeta(1,1)
p7 ~ dbeta(1,1)
……
……
……
p673 ~ dbeta(1,1)

```
p<-p1
se1od1<-p2
se1od2<-p16
se1od3<-p30
se1od4<-p44
se1od5<-p58
se1od6<-p72
se1od7<-p86
se1od8<-p100
se1od9<-p114
se1od10<-p128
se1od11<-p142
se1od12<-p156
se1od13<-p170
se1od14<-p184
se1od15<-p198
se1od16<-p212
se1od17<-p226
se1od18<-p240
se1od19<-p254
se1od20<-p268
se1od21<-p282
se1od22<-p296
se1od23<-p310
se1od24<-p324
se1od25<-p338
se1od26<-p352
se1od27<-p366
se1od28<-p380
se1od29<-p394
se1od30<-p408
se1od31<-p422
se1od32<-p436
```

```
se1od33<-p450
se1od34<-p464
se1od35<-p478
se1od36<-p492
se1od37<-p506
se1od38<-p520
se1od39<-p534
se1od40<-p548
se1od41<-p562
se1od42<-p576
se1od43<-p590
se1od44<-p604
se1od45<-p618
se1od46<-p632
se1od47<-p646
se1od48<-p660
sp1od1<-p3
sp1od2<-p17
sp1od3<-p31
sp1od4<-p45
sp1od5<-p59
sp1od6<-p73
sp1od7<-p87
sp1od8<-p101
sp1od9<-p115
sp1od10<-p129
sp1od11<-p143
sp1od12<-p157
sp1od13<-p171
sp1od14<-p185
sp1od15<-p199
sp1od16<-p213
sp1od17<-p227
sp1od18<-p241
sp1od19<-p255
sp1od20<-p269
sp1od21<-p283
sp1od22<-p297
sp1od23<-p311
sp1od24<-p325
sp1od25<-p339
sp1od26<-p353
sp1od27<-p367
sp1od28<-p381
sp1od29<-p395
sp1od30<-p409
sp1od31<-p423
sp1od32<-p437
sp1od33<-p451
sp1od34<-p465
sp1od35<-p479
sp1od36<-p493
sp1od37<-p507
sp1od38<-p521
sp1od39<-p535
sp1od40<-p549
sp1od41<-p563
sp1od42<-p577
sp1od43<-p591
sp1od44<-p605
sp1od45<-p619
```

```
sp1od46<-p633
sp1od47<-p647
sp1od48<-p661
se2od1<-p2*p4+(1-p2)*p5
se2od2<-p16*p18+(1-p16)*p19
se2od3<-p30*p32+(1-p30)*p33
se2od4<-p44*p46+(1-p44)*p47
se2od5<-p58*p60+(1-p58)*p61
se2od6<-p72*p74+(1-p72)*p75
se2od7<-p86*p88+(1-p86)*p89
se2od8<-p100*p102+(1-p100)*p103
se2od9<-p114*p116+(1-p114)*p117
se2od10<-p128*p130+(1-p128)*p131
se2od11<-p142*p144+(1-p142)*p145
se2od12<-p156*p158+(1-p156)*p159
se2od13<-p170*p172+(1-p170)*p173
se2od14<-p184*p186+(1-p184)*p187
se2od15<-p198*p200+(1-p198)*p201
se2od16<-p212*p214+(1-p212)*p215
se2od17<-p226*p228+(1-p226)*p229
se2od18<-p240*p242+(1-p240)*p243
se2od19<-p254*p256+(1-p254)*p257
se2od20<-p268*p270+(1-p268)*p271
se2od21<-p282*p284+(1-p282)*p285
se2od22<-p296*p298+(1-p296)*p299
se2od23<-p310*p312+(1-p310)*p313
se2od24<-p324*p326+(1-p324)*p327
se2od25<-p338*p340+(1-p338)*p341
se2od26<-p352*p354+(1-p352)*p355
se2od27<-p366*p368+(1-p366)*p369
se2od28<-p380*p382+(1-p380)*p383
se2od29<-p394*p396+(1-p394)*p397
se2od30<-p408*p410+(1-p408)*p411
se2od31<-p422*p424+(1-p422)*p425
se2od32<-p436*p438+(1-p436)*p439
se2od33<-p450*p452+(1-p450)*p453
se2od34<-p464*p466+(1-p464)*p467
se2od35<-p478*p480+(1-p478)*p481
se2od36<-p492*p494+(1-p492)*p495
se2od37<-p506*p508+(1-p506)*p509
se2od38<-p520*p522+(1-p520)*p523
se2od39<-p534*p536+(1-p534)*p537
se2od40<-p548*p550+(1-p548)*p551
se2od41<-p562*p564+(1-p562)*p565
se2od42<-p576*p578+(1-p576)*p579
se2od43<-p590*p592+(1-p590)*p593
se2od44<-p604*p606+(1-p604)*p607
se2od45<-p618*p620+(1-p618)*p621
se2od46<-p632*p634+(1-p632)*p635
se2od47<-p646*p648+(1-p646)*p649
se2od48<-p660*p662+(1-p660)*p663

sp2od1<-p3*p6+(1-p3)*p7
sp2od2<-p17*p20+(1-p17)*p21
sp2od3<-p31*p34+(1-p31)*p35
sp2od4<-p45*p48+(1-p45)*p49
sp2od5<-p59*p62+(1-p59)*p63
sp2od6<-p73*p76+(1-p73)*p77
sp2od7<-p87*p90+(1-p87)*p91
sp2od8<-p101*p104+(1-p101)*p105
sp2od9<-p115*p118+(1-p115)*p119
```

```
sp2od10<-p129*p132+(1-p129)*p133
sp2od11<-p143*p146+(1-p143)*p147
sp2od12<-p157*p160+(1-p157)*p161
sp2od13<-p171*p174+(1-p171)*p175
sp2od14<-p185*p188+(1-p185)*p189
sp2od15<-p199*p202+(1-p199)*p203
sp2od16<-p213*p216+(1-p213)*p217
sp2od17<-p227*p230+(1-p227)*p231
sp2od18<-p241*p244+(1-p241)*p245
sp2od19<-p255*p258+(1-p255)*p259
sp2od20<-p269*p272+(1-p269)*p273
sp2od21<-p283*p286+(1-p283)*p287
sp2od22<-p297*p300+(1-p297)*p301
sp2od23<-p311*p314+(1-p311)*p315
sp2od24<-p325*p328+(1-p325)*p329
sp2od25<-p339*p342+(1-p339)*p343
sp2od26<-p353*p356+(1-p353)*p357
sp2od27<-p367*p370+(1-p367)*p371
sp2od28<-p381*p374+(1-p381)*p385
sp2od29<-p395*p398+(1-p395)*p399
sp2od30<-p409*p412+(1-p409)*p413
sp2od31<-p423*p426+(1-p423)*p427
sp2od32<-p437*p440+(1-p437)*p441
sp2od33<-p451*p454+(1-p451)*p455
sp2od34<-p465*p468+(1-p465)*p469
sp2od35<-p479*p482+(1-p479)*p483
sp2od36<-p493*p496+(1-p493)*p497
sp2od37<-p507*p510+(1-p507)*p511
sp2od38<-p521*p524+(1-p521)*p525
sp2od39<-p535*p538+(1-p535)*p539
sp2od40<-p549*p552+(1-p549)*p553
sp2od41<-p563*p566+(1-p563)*p567
sp2od42<-p577*p580+(1-p577)*p581
sp2od43<-p591*p594+(1-p591)*p595
sp2od44<-p605*p608+(1-p605)*p609
sp2od45<-p619*p622+(1-p619)*p623
sp2od46<-p633*p636+(1-p633)*p637
sp2od47<-p647*p650+(1-p647)*p651
sp2od48<-p661*p664+(1-p661)*p665

se3od1<-p2*(p4*p8+(1-p4)*p9)+(1-p2)*(p5*p10+(1-p5)*p11)
se3od2<-p16*(p18*p22+(1-p18)*p23)+(1-p16)*(p19*p24+(1-p19)*p25)
se3od3<-p30*(p32*p36+(1-p32)*p37)+(1-p30)*(p33*p38+(1-p33)*p39)
se3od4<-p44*(p46*p50+(1-p46)*p51)+(1-p44)*(p47*p52+(1-p47)*p53)
se3od5<-p58*(p60*p64+(1-p60)*p65)+(1-p58)*(p61*p66+(1-p61)*p67)
se3od6<-p72*(p74*p78+(1-p74)*p79)+(1-p72)*(p75*p80+(1-p75)*p81)
se3od7<-p86*(p88*p92+(1-p88)*p93)+(1-p86)*(p89*p94+(1-p89)*p95)
se3od8<-p100*(p102*p106+(1-p102)*p107)+(1-p100)*(p103*p108+(1-p103)*p109)
se3od9<-p114*(p116*p120+(1-p116)*p121)+(1-p114)*(p117*p122+(1-p117)*p123)
se3od10<-p128*(p130*p134+(1-p130)*p135)+(1-p128)*(p131*p136+(1-p131)*p137)
se3od11<-p142*(p144*p148+(1-p144)*p149)+(1-p142)*(p145*p150+(1-p145)*p151)
se3od12<-p156*(p158*p162+(1-p158)*p163)+(1-p156)*(p159*p164+(1-p159)*p165)
se3od13<-p170*(p172*p176+(1-p172)*p177)+(1-p170)*(p173*p178+(1-p173)*p179)
se3od14<-p184*(p186*p190+(1-p186)*p191)+(1-p184)*(p187*p192+(1-p187)*p193)
se3od15<-p198*(p200*p204+(1-p200)*p205)+(1-p198)*(p201*p206+(1-p201)*p207)
se3od16<-p212*(p214*p218+(1-p214)*p219)+(1-p212)*(p215*p220+(1-p215)*p221)
se3od17<-p226*(p228*p232+(1-p228)*p233)+(1-p226)*(p229*p234+(1-p229)*p235)
se3od18<-p240*(p242*p246+(1-p242)*p247)+(1-p240)*(p243*p248+(1-p243)*p249)
se3od19<-p254*(p256*p260+(1-p256)*p261)+(1-p254)*(p257*p262+(1-p257)*p263)
se3od20<-p268*(p270*p274+(1-p270)*p275)+(1-p268)*(p271*p276+(1-p271)*p277)
se3od21<-p282*(p284*p288+(1-p284)*p289)+(1-p282)*(p285*p290+(1-p285)*p291)
```

```
se3od22<-p296*(p298*p302+(1-p298)*p203)+(1-p296)*(p299*p304+(1-p299)*p305)
se3od23<-p310*(p312*p316+(1-p312)*p317)+(1-p310)*(p313*p318+(1-p313)*p319)
se3od24<-p324*(p326*p330+(1-p326)*p331)+(1-p324)*(p327*p332+(1-p327)*p333)
se3od25<-p338*(p340*p344+(1-p340)*p345)+(1-p338)*(p341*p346+(1-p341)*p347)
se3od26<-p352*(p354*p358+(1-p354)*p359)+(1-p352)*(p355*p360+(1-p355)*p361)
se3od27<-p366*(p368*p372+(1-p368)*p373)+(1-p366)*(p369*p374+(1-p369)*p375)
se3od28<-p380*(p382*p386+(1-p382)*p387)+(1-p380)*(p383*p388+(1-p383)*p389)
se3od29<-p394*(p396*p400+(1-p396)*p401)+(1-p394)*(p397*p402+(1-p397)*p403)
se3od30<-p408*(p410*p414+(1-p410)*p415)+(1-p408)*(p411*p416+(1-p411)*p417)
se3od31<-p422*(p424*p428+(1-p424)*p429)+(1-p422)*(p425*p430+(1-p425)*p431)
se3od32<-p436*(p438*p442+(1-p438)*p443)+(1-p436)*(p439*p444+(1-p439)*p445)
se3od33<-p450*(p452*p456+(1-p452)*p457)+(1-p450)*(p453*p458+(1-p453)*p459)
se3od34<-p464*(p466*p470+(1-p466)*p471)+(1-p464)*(p467*p472+(1-p467)*p473)
se3od35<-p478*(p480*p484+(1-p480)*p485)+(1-p478)*(p481*p486+(1-p481)*p487)
se3od36<-p492*(p494*p498+(1-p494)*p499)+(1-p492)*(p495*p500+(1-p495)*p501)
se3od37<-p506*(p508*p512+(1-p508)*p513)+(1-p506)*(p509*p514+(1-p509)*p515)
se3od38<-p520*(p522*p526+(1-p522)*p527)+(1-p520)*(p523*p528+(1-p523)*p529)
se3od39<-p534*(p536*p540+(1-p536)*p541)+(1-p534)*(p537*p542+(1-p537)*p543)
se3od40<-p548*(p550*p554+(1-p550)*p555)+(1-p548)*(p551*p556+(1-p551)*p557)
se3od41<-p562*(p564*p568+(1-p564)*p569)+(1-p562)*(p565*p570+(1-p565)*p571)
se3od42<-p576*(p578*p582+(1-p578)*p583)+(1-p576)*(p579*p584+(1-p579)*p585)
se3od43<-p590*(p592*p596+(1-p592)*p597)+(1-p590)*(p593*p598+(1-p593)*p599)
se3od44<-p604*(p606*p610+(1-p606)*p611)+(1-p604)*(p607*p612+(1-p607)*p613)
se3od45<-p618*(p620*p624+(1-p620)*p625)+(1-p618)*(p621*p626+(1-p621)*p627)
se3od46<-p632*(p634*p638+(1-p634)*p639)+(1-p632)*(p635*p640+(1-p635)*p641)
se3od47<-p646*(p648*p652+(1-p648)*p653)+(1-p646)*(p649*p654+(1-p649)*p655)
se3od48<-p660*(p662*p666+(1-p662)*p667)+(1-p660)*(p663*p668+(1-p663)*p669)


sp3od1<-p3*(p6*p12+(1-p6)*p13)+(1-p3)*(p7*p14+(1-p7)*p15)
sp3od2<-p17*(p20*p26+(1-p20)*p27)+(1-p17)*(p21*p28+(1-p21)*p29)
sp3od3<-p31*(p34*p40+(1-p34)*p41)+(1-p31)*(p35*p42+(1-p35)*p43)
sp3od4<-p45*(p48*p54+(1-p48)*p55)+(1-p45)*(p49*p56+(1-p49)*p57)
sp3od5<-p59*(p62*p68+(1-p62)*p69)+(1-p59)*(p63*p70+(1-p63)*p71)
sp3od6<-p73*(p76*p82+(1-p76)*p83)+(1-p73)*(p77*p84+(1-p77)*p85)
sp3od7<-p87*(p90*p96+(1-p90)*p97)+(1-p87)*(p91*p98+(1-p91)*p99)
sp3od8<-p101*(p104*p110+(1-p104)*p111)+(1-p101)*(p105*p112+(1-p105)*p113)
sp3od9<-p115*(p118*p124+(1-p118)*p125)+(1-p115)*(p119*p126+(1-p119)*p127)
sp3od10<-p129*(p132*p138+(1-p132)*p139)+(1-p129)*(p133*p140+(1-p133)*p141)
sp3od11<-p143*(p146*p152+(1-p146)*p153)+(1-p143)*(p147*p154+(1-p147)*p155)
sp3od12<-p157*(p160*p166+(1-p160)*p167)+(1-p157)*(p161*p168+(1-p161)*p169)
sp3od13<-p171*(p174*p180+(1-p174)*p181)+(1-p171)*(p175*p182+(1-p175)*p183)
sp3od14<-p185*(p188*p194+(1-p188)*p195)+(1-p185)*(p189*p196+(1-p189)*p197)
sp3od15<-p199*(p202*p208+(1-p202)*p209)+(1-p199)*(p203*p210+(1-p203)*p211)
sp3od16<-p213*(p216*p222+(1-p216)*p223)+(1-p213)*(p217*p224+(1-p217)*p225)
sp3od17<-p227*(p230*p236+(1-p230)*p237)+(1-p227)*(p231*p238+(1-p231)*p239)
sp3od18<-p241*(p244*p250+(1-p244)*p251)+(1-p241)*(p245*p252+(1-p245)*p253)
sp3od19<-p255*(p258*p264+(1-p258)*p265)+(1-p255)*(p259*p266+(1-p259)*p267)
sp3od20<-p269*(p272*p278+(1-p272)*p279)+(1-p269)*(p273*p280+(1-p273)*p281)
sp3od21<-p283*(p286*p292+(1-p286)*p293)+(1-p283)*(p287*p294+(1-p287)*p295)
sp3od22<-p297*(p300*p306+(1-p300)*p307)+(1-p297)*(p301*p308+(1-p301)*p309)
sp3od23<-p311*(p314*p320+(1-p314)*p321)+(1-p311)*(p315*p322+(1-p315)*p323)
sp3od24<-p325*(p328*p334+(1-p328)*p335)+(1-p325)*(p329*p336+(1-p329)*p337)
sp3od25<-p339*(p342*p348+(1-p342)*p349)+(1-p339)*(p343*p350+(1-p343)*p351)
sp3od26<-p353*(p356*p362+(1-p356)*p363)+(1-p353)*(p357*p364+(1-p357)*p365)
sp3od27<-p367*(p370*p376+(1-p370)*p377)+(1-p367)*(p371*p378+(1-p371)*p379)
sp3od28<-p381*(p384*p390+(1-p384)*p391)+(1-p381)*(p385*p392+(1-p385)*p393)
sp3od29<-p395*(p398*p404+(1-p398)*p405)+(1-p395)*(p399*p406+(1-p399)*p407)
sp3od30<-p409*(p412*p418+(1-p412)*p419)+(1-p409)*(p413*p420+(1-p413)*p421)
sp3od31<-p423*(p426*p432+(1-p426)*p433)+(1-p423)*(p427*p434+(1-p427)*p435)
sp3od32<-p437*(p440*p446+(1-p440)*p447)+(1-p437)*(p441*p448+(1-p441)*p449)
```

```
sp3od33<-p451*(p454*p460+(1-p454)*p461)+(1-p451)*(p455*p462+(1-p455)*p463)
sp3od34<-p465*(p468*p474+(1-p468)*p475)+(1-p465)*(p469*p476+(1-p469)*p477)
sp3od35<-p479*(p482*p488+(1-p482)*p489)+(1-p479)*(p483*p490+(1-p483)*p491)
sp3od36<-p493*(p496*p502+(1-p496)*p503)+(1-p493)*(p497*p504+(1-p497)*p505)
sp3od37<-p507*(p510*p516+(1-p510)*p517)+(1-p507)*(p511*p518+(1-p511)*p519)
sp3od38<-p521*(p524*p530+(1-p524)*p531)+(1-p521)*(p525*p532+(1-p525)*p533)
sp3od39<-p535*(p538*p544+(1-p538)*p545)+(1-p535)*(p539*p546+(1-p539)*p547)
sp3od40<-p549*(p552*p558+(1-p552)*p559)+(1-p549)*(p553*p560+(1-p553)*p561)
sp3od41<-p563*(p566*p572+(1-p566)*p573)+(1-p563)*(p567*p574+(1-p567)*p575)
sp3od42<-p577*(p580*p586+(1-p580)*p587)+(1-p577)*(p581*p588+(1-p581)*p589)
sp3od43<-p591*(p594*p600+(1-p594)*p601)+(1-p591)*(p595*p602+(1-p595)*p603)
sp3od44<-p605*(p608*p614+(1-p608)*p615)+(1-p605)*(p609*p616+(1-p609)*p617)
sp3od45<-p619*(p622*p628+(1-p622)*p629)+(1-p619)*(p623*p630+(1-p623)*p631)
sp3od46<-p633*(p636*p642+(1-p636)*p643)+(1-p633)*(p637*p644+(1-p637)*p645)
sp3od47<-p647*(p650*p656+(1-p650)*p657)+(1-p647)*(p651*p658+(1-p651)*p659)
sp3od48<-p661*(p664*p670+(1-p664)*p671)+(1-p661)*(p665*p672+(1-p665)*p673)

      }
```

list(n=66, rod1=c(19, 0,15,0,13,0,19,0), rod2=c(19,0,14,1,13,0,19,0),
rod3=c(19,0,12,3,13,0,19,0), rod4=c(19,0,11,4,13,0,18,1), rod5=c(19,0,10,5,13,0,17,2),
rod6=c(19,0,10,5,13,0,16,3), rod7=c(19,0,9,6,13,0,16,3), rod8=c(19,0,9,6,13,0,15,4),
rod9=c(19,0,9,6,13,0,14,5), rod10=c(19,0,8,7,13,0,14,5), rod11=c(19,0,8,7,13,0,13,6),
rod12=c(19,0,8,7,13,0,12,7), rod13=c(19,0,8,7,13,0,11,8), rod14=c(18,1,8,7,13,0,11,8),
rod15=c(18,1,8,7,13,0,10,9), rod16=c(17,2,8,7,13,0,10,9), rod17=c(15,4,8,7,13,0,10,9),
rod18=c(15,4,7,8,13,0,9,10), rod19=c(15,4,6,9,13,0,9,10), rod20=c(15,4,7,8,13,0,9,10),
rod21=c(14,5,7,8,13,0,9,10), rod22=c(13,6,7,8,13,0,8,11), rod23=c(13,6,7,8,12,1,7,12),
rod24=c(13,6,6,9,12,1,6,13), rod25=c(13,6,4,11,12,1,6,13), rod26=c(12,7,3,12,12,1,6,13),
rod27=c(11,8,3,12,12,1,6,13), rod28=c(11,8,2,13,11,2,6,13), rod29=c(10,9,2,13,11,2,6,13),
rod30=c(9,12,2,13,11,2,5,12), rod31=c(9,10,2,13,10,3,5,14), rod32=c(8,11,2,13,10,3,5,14),
rod33=c(7,12,2,13,8,5,4,15), rod34=c(6,13,1,14,8,5,4,15), rod35=c(5,14,1,14,8,5,4,15),
rod36=c(4,15,0,15,8,5,4,15), rod37=c(4,15,0,15,8,5,2,17), rod38=c(4,15,0,15,8,5,1,18),
rod39=c(4,15,0,15,7,6,1,18), rod40=c(3,16,0,15,7,6,1,18), rod41=c(2,17,0,15,7,6,0,19),
rod42=c(2,17,0,15,6,7,0,19), rod43=c(1,18,0,15,6,7,0,19), rod44=c(0,19,0,15,5,8,0,19),
rod45=c(0,19,1,14,4,9,0,19), rod46=c(0,19,1,14,3,10,0,19), rod47=c(0,19,1,14,3,10,0,19),
rod48=c(0,19,0,15,1,12,0,19))

Appendix 3.3. Comparison of non-parametric ROC analysis and Bayesian ROC analysis (informative and non-informative opinion) of ELISA results obtained with serum samples from Zambian pigs

Cut-0ff-ELISA (OD)	NON PARAMETRIC ROC ANALYSIS		BAYESIAN ROC APPROACH ANALYSIS							
			Informative opinion				Non informative opinion			
	Se(%)	Sp(%)	Se(%)	95 %CI	Sp(%)	95 %CI	Se	95 % CI	Sp	95 % CI
≥0.16	100	0	94	87;100	5	1 ; 10	92	82 ; 98	08	2 ; 19
≥0.17	100	3	93	86 ;.98	7	2 ; 13	91	79 ; 98	09	2 ; 21
≥0.19	96	5	93	85 ; 98	10	4 ; 17	88	75 ; 97	12	3 ; 26
≥0.20	96	10	92	84 ; 98	14	7 ; 23	85	70 ; 96	15	4 ; 30
≥0.22	93	13	89	80 ; 96	17	9 ; 26	83	66 ; 95	17	5 ; 34
≥0.25	93	15	87	77 ; 95	19	10 ; 28	81	64 ; 94	18	5 ; 36
≥0.26	89	16	84	73 ; .93	19	10 ; 29	80	62 ; 94	20	6 ; 38
≥0.28	86	16	82	70 ; 91	19	10 ; 29	79	60 ; 94	21	6 ; 40
≥0.29	86	18	79	67 ; 89	21	12 ; 31	78	58 ; 93	22	7 ; 42
≥0.31	86	21	82	70 ; 91	24	14 ; 34	76	56 ; 93	24	7 ; 44
≥0.32	86	24	81	69 ; 91	26	16 ; 37	75	55; 92	25	8 ; 46
≥0.37	82	24	79	66 ; 89	26	16 ; 37	74	52 ; 92	26	8 ; 48
≥0.38	82	26	79	66 ; 89	29	18 ; 40	73	50 ; 92	28	9 ; 50
≥0.43	79	26	76	63 ; 87	29	19 ; 41	71	49 ; 90	29	10 ; 51
≥0.44	75	26	73	60 ; 85	29	19 ; 41	70	47 ; 90	30	10 ; 53
≥0.46	71	26	70	57 ; 82	29	19 ; 41	69	46 ; 89	31	11 ; 55
≥0.47	68	29	66	53 ; 79	31	20 ; 43	66	43 ; 86	34	13 ; 57
≥0.52	64	32	64	49 ; 77	34	23 ; 46	64	40 ; 85	36	14 ; 60
≥0.53	61	32	61	47 ; 74	34	23 ; 46	63	38 ; 85	38	15 ; 62
≥0.57	61	34	64	49 ; 77	34	23 ; 45	64	39 ; 86	36	14 ; 61
≥0.59	57	34	60	46 ; 74	34	23 ; 46	63	38 ; 85	38	16 ; 62
≥0.60	54	37	60	46 ; 72	36	25 ; 48	60	34 ; 84	40	17 ; 65
≥0.61	55	42	57	43 ; 71	41	29 ; 53	58	33 ; 81	43	19 ; 67
≥0.63	57	47	57	43 ; 70	46	34 ; 58	55	30 ; 79	45	21 ; 71
≥0.65	50	47	54	39 ; 67	48	36 ; 60	52	26 ; 78	48	22 ; 74
≥0.68	46	50	51	36 ; 65	50	38 ; 62	50	25 ; 76	50	24 ; 76
≥0.69	43	50	47	33 ; 61	50	38 ; 62	49	23 ; 74	51	25 ; 77
≥0.71	39	53	44	31 ; 58	53	41 ; 65	46	21 ; 72	54	28 ; 79
≥0.73	36	53	41	28 ; 55	53	41 ; 65	45	20 ; 71	55	30 ; 80
≥0.74	36	55	37	24 ; 51	55	42 ; 66	43	18 ; 68	58	32 ; 82
≥0.75	36	58	38	25 ; 52	58	46 ; 69	41	18 ; 66	59	34 ; 82
≥0.78	36	60	37	24 ; 52	60	48 ; 71	40	17 ; 65	60	35 ; 83
≥0.79	32	68	37	24 ; 51	67	55 ; 78	36	15 ; 60	64	37 ; 86
≥0.80	25	68	27	15 ; 40	67	55 ; 78	33	13 ; 56	68	45 ; 88
≥0.82	25	71	27	15 ; 40	69	58 ; 80	31	12 ; 54	69	47 ; 89
≥0.87	21	74	25	14 ; 38	71	59 ; 82	29	9 ; 52	67	31 ; 92
≥0.89	21	79	24	13 ; 37	76	65 ; 86	26	8 ; 48	74	52 ; 92
≥0.90	21	82	24	13 ; 37	78	68 ; 88	25	8 ; 47	75	54 ; 93
≥0.92	18	82	22	11 ; 34	79	68 ; 88	24	7 ; 44	77	56 ; 93
≥1.02	14	82	19	9 ; 31	79	68 ; 88	22	7 ; 42	78	57 ; 93
≥1.03	11	84	15	7 ; 27	81	71 ; 90	20	5 ; 40	81	61 ; 95
≥1.04	11	87	15	7 ; 26	83	74 ; 91	18	5 ; 37	82	63 ; 95
≥1.14	11	89	14	6 ; 25	85	76 ; 93	17	4 ; 35	83	65 ; 96
≥1.18	7	92	12	4 ; 22	88	79 ; 95	14	3 ; 31	85	68 ; 97
≥1.47	7	95	12	5 ; 22	88	80 ; 95	15	4 ; 30	86	70 ; 96
≥1.64	7	97	12	5 ; 21	90	83 ; 96	13	4 ; 28	87	72 ; 97
≥1.75	3	97	12	5 ; 21	90	83 ; 96	13	3 ; 27	87	72 ; 96
≥1.76	3	100	08	3 ; 16	95	89 ;100	9	2 ; 21	91	79 ; 98

4. Conditional independence model

Two tests are conditionally independent when the sensitivity (or specificity) of the second test (T2) does not depend on whether the results of the first test (T1) are positive or negative for infected or non-infected animals (Gardner et *al*., 2000). In this model we used tongue inspection and Ab-ELISA-F3 as T1 and T2 respectively.
Assume that the two tests are applied on infected (D^+) and non-infected (D^-) groups of pigs. At a selected cut-off, for example, the probability of an individual from the infected group to test positive on both tests is

$P11 = P(T1^+ , T2^+)=P(T1^+ , T2^+ / D^+)P(D^+)+P(T1^+ , T2^+ / D^-)P(D^-)$

$= p*se1*se2+(1-p)*(1-sp1)*(1-se2)$

where se1= sensitivity of T1, sp1 = specificity of T1, se2 = sensitivity of T2 and sp= specificity of T2.
The probabilities of the other possible results of the two tests are computed in a similar manner.
Example: the probabilities of possible results at 5 possible cut-off are given as follows:

od1 : P00 = p*(1-se1)*(1-se2) + (1-pi)*sp1*sp2
od1 : P01 = p*(1-se1)*se2 + (1-p)*sp1*(1-sp2)
od1 : P10 = p*se1*(1-se2) + (1-p)*(1-sp1)*sp2
od1 : P11 = p*se1*se2+ (1-p)*(1-sp1)*(1-sp2)
od2 : P00 = p*(1-se3)*(1-se4) + (1-p)*sp3*sp4
od2 : P01 = p*(1-se3)*se4 + (1-p)*sp3*(1-sp4)
od2 : P10 = p*se3*(1-se4) + (1-p)*(1-sp3)*sp4
od2 : P11 = p*se3*se4+ (1-p)*(1-sp3)*(1-sp4)
od3 : P00 = p*(1-se5)*(1-se6) + (1-p)*sp5*sp6
od3 : P01 = p*(1-se5)*se6 + (1-p)*sp5*(1-sp6)
od3 : P10 = p*se5*(1-se6) + (1-p)*(1-sp5)*sp6
od3 : P11 = p*se5*se6+ (1-p)*(1-sp5)*(1-sp6)
od4 : P00 = p*(1-se7)*(1-se8) + (1-p)*sp7*sp8
od4 : P01 = p*(1-se7)*se8 + (1-p)*sp7*(1-sp8)
od4 : P10 = p*se7*(1-se8) + (1-p)*(1-sp7)*sp8
od4 : P11 = p*se7*se8+ (1-p)*(1-sp7)*(1-sp8)
od5 : P00 = p*(1-se9)*(1-se10) + (1-p)*sp9*sp10
od5 : P01 = p*(1-se9)*se10 + (1-p)*sp9*(1-sp10)
od5 : P10 = p*se9*(1-se10) + (1-p)*(1-sp9)*sp10
od5 : P11 = p*se9*se10+ (1-p)*(1-sp9)*(1-sp10)

WinBUGS code is given in **Appendix 4.1.** The model was run without expert opinion and the specificity of tongue inspection is equal to 1.

Appendix 4.1.WinBUGS code for conditional independence model using serum samples of pigs from Cameroon (40 different optical densities)

```
model
{
rod1[1:4] ~ dmulti(prod1[1:4], n)
rod2[1:4] ~ dmulti(prod2[1:4], n)
rod3[1:4] ~ dmulti(prod3[1:4], n)
rod4[1:4] ~ dmulti(prod4[1:4], n)
rod5[1:4] ~ dmulti(prod5[1:4], n)
rod6[1:4] ~ dmulti(prod6[1:4], n)
rod7[1:4] ~ dmulti(prod7[1:4], n)
rod8[1:4] ~ dmulti(prod8[1:4], n)
rod9[1:4] ~ dmulti(prod9[1:4], n)
rod10[1:4] ~ dmulti(prod10[1:4], n)
rod11[1:4] ~ dmulti(prod11[1:4], n)
rod12[1:4] ~ dmulti(prod12[1:4], n)
rod13[1:4] ~ dmulti(prod13[1:4], n)
rod14[1:4] ~ dmulti(prod14[1:4], n)
rod15[1:4] ~ dmulti(prod15[1:4], n)
rod16[1:4] ~ dmulti(prod16[1:4], n)
rod17[1:4] ~ dmulti(prod17[1:4], n)
rod18[1:4] ~ dmulti(prod18[1:4], n)
rod19[1:4] ~ dmulti(prod19[1:4], n)
rod20[1:4] ~ dmulti(prod20[1:4], n)
rod21[1:4] ~ dmulti(prod21[1:4], n)
rod22[1:4] ~ dmulti(prod22[1:4], n)
rod23[1:4] ~ dmulti(prod23[1:4], n)
rod24[1:4] ~ dmulti(prod24[1:4], n)
rod25[1:4] ~ dmulti(prod25[1:4], n)
rod26[1:4] ~ dmulti(prod26[1:4], n)
rod27[1:4] ~ dmulti(prod27[1:4], n)
rod28[1:4] ~ dmulti(prod28[1:4], n)
rod29[1:4] ~ dmulti(prod29[1:4], n)
rod30[1:4] ~ dmulti(prod30[1:4], n)
rod31[1:4] ~ dmulti(prod31[1:4], n)
rod32[1:4] ~ dmulti(prod32[1:4], n)
rod33[1:4] ~ dmulti(prod33[1:4], n)
rod34[1:4] ~ dmulti(prod34[1:4], n)
rod35[1:4] ~ dmulti(prod35[1:4], n)
rod36[1:4] ~ dmulti(prod36[1:4], n)
rod37[1:4] ~ dmulti(prod37[1:4], n)
rod38[1:4] ~ dmulti(prod38[1:4], n)
rod39[1:4] ~ dmulti(prod39[1:4], n)
rod40[1:4] ~ dmulti(prod40[1:4], n)

        prod1[1] <- p*(1-se1)*(1-se2) + (1-p)*sp2
        prod1[2] <- p*(1-se1)*se2 + (1-p)*(1-sp2)
        prod1[3] <- p*se1*(1-se2)
        prod1[4] <- p*se1*se2
        prod2[1] <- p*(1-se3)*(1-se4) + (1-p)*sp4
        prod2[2] <- p*(1-se3)*se4 + (1-p)*(1-sp4)
        prod2[3] <- p*se3*(1-se4)
        prod2[4] <- p*se3*se4
        prod3[1] <- p*(1-se5)*(1-se6) + (1-p)*sp6
        prod3[2] <- p*(1-se5)*se6 + (1-p)*(1-sp6)
        prod3[3] <- p*se5*(1-se6)
        prod3[4] <- p*se5*se6
        prod4[1] <- p*(1-se7)*(1-se8) + (1-p)*sp8
```

```
prod4[2] <- p*(1-se7)*se8 + (1-p)*(1-sp8)
prod4[3] <- p*se7*(1-se8)
prod4[4] <- p*se7*se8
prod5[1] <- p*(1-se9)*(1-se10) + (1-p)*sp10
prod5[2] <- p*(1-se9)*se10 + (1-p)*(1-sp10)
prod5[3] <- p*se9*(1-se10)
prod5[4] <- p*se9*se10
prod6[1] <- p*(1-se11)*(1-se12) + (1-p)*sp12
prod6[2] <- p*(1-se11)*se12 + (1-p)*(1-sp12)
prod6[3] <- p*se11*(1-se12)
prod6[4] <- p*se11*se12
prod7[1] <- p*(1-se13)*(1-se14) + (1-p)*sp14
prod7[2] <- p*(1-se13)*se14 + (1-p)*(1-sp14)
prod7[3] <- p*se13*(1-se14)
prod7[4] <- p*se13*se14
prod8[1] <- p*(1-se15)*(1-se16) + (1-p)*sp16
prod8[2] <- p*(1-se15)*se16 + (1-p)*(1-sp16)
prod8[3] <- p*se15*(1-se16)
prod8[4] <- p*se15*se16
prod9[1] <- p*(1-se17)*(1-se18) + (1-p)*sp18
prod9[2] <- p*(1-se17)*se18 + (1-p)*(1-sp18)
prod9[3] <- p*se17*(1-se18)
prod9[4] <- p*se17*se18
prod10[1] <- p*(1-se19)*(1-se20) + (1-p)*sp20
prod10[2] <- p*(1-se19)*se20 + (1-p)*(1-sp20)
prod10[3] <- p*se19*(1-se20)
prod10[4] <- p*se19*se20
prod11[1] <- p*(1-se21)*(1-se22) + (1-p)*sp22
prod11[2] <- p*(1-se21)*se22 + (1-p)*(1-sp22)
prod11[3] <- p*se21*(1-se22)
prod11[4] <- p*se21*se22
prod12[1] <- p*(1-se23)*(1-se24) + (1-p)*sp24
prod12[2] <- p*(1-se23)*se24 + (1-p)*(1-sp24)
prod12[3] <- p*se23*(1-se24)
prod12[4] <- p*se23*se24
prod13[1] <- p*(1-se25)*(1-se26) + (1-p)*sp26
prod13[2] <- p*(1-se25)*se26 + (1-p)*(1-sp26)
prod13[3] <- p*se25*(1-se26)
prod13[4] <- p*se25*se26
prod14[1] <- p*(1-se27)*(1-se28) + (1-p)*sp28
prod14[2] <- p*(1-se27)*se28 + (1-p)*(1-sp28)
prod14[3] <- p*se27*(1-se28)
prod14[4] <- p*se27*se28
prod15[1] <- p*(1-se29)*(1-se30) + (1-p)*sp30
prod15[2] <- p*(1-se29)*se30 + (1-p)*(1-sp30)
prod15[3] <- p*se29*(1-se30)
prod15[4] <- p*se29*se30
prod16[1] <- p*(1-se31)*(1-se32) + (1-p)*sp32
prod16[2] <- p*(1-se31)*se32 + (1-p)*(1-sp32)
prod16[3] <- p*se31*(1-se32)
prod16[4] <- p*se31*se32
prod17[1] <- p*(1-se33)*(1-se34) + (1-p)*sp34
prod17[2] <- p*(1-se33)*se34 + (1-p)*(1-sp34)
prod17[3] <- p*se33*(1-se34)
prod17[4] <- p*se33*se34
prod18[1] <- p*(1-se35)*(1-se36) + (1-p)*sp36
prod18[2] <- p*(1-se35)*se36 + (1-p)*(1-sp36)
prod18[3] <- p*se35*(1-se36)
prod18[4] <- p*se35*se36
prod19[1] <- p*(1-se37)*(1-se38) + (1-p)*sp38
```

```
prod19[2] <- p*(1-se37)*se38 + (1-p)*(1-sp38)
prod19[3] <- p*se37*(1-se38)
prod19[4] <- p*se37*se38
prod20[1] <- p*(1-se39)*(1-se40) + (1-p)*sp40
prod20[2] <- p*(1-se39)*se40 + (1-p)*(1-sp40)
prod20[3] <- p*se39*(1-se40)
prod20[4] <- p*se39*se40
prod21[1] <- p*(1-se41)*(1-se42) + (1-p)*sp42
prod21[2] <- p*(1-se41)*se42 + (1-p)*(1-sp42)
prod21[3] <- p*se41*(1-se42)
prod21[4] <- p*se41*se42
prod22[1] <- p*(1-se43)*(1-se44) + (1-p)*sp44
prod22[2] <- p*(1-se43)*se44 + (1-p)*(1-sp44)
prod22[3] <- p*se43*(1-se44)
prod22[4] <- p*se43*se44
prod23[1] <- p*(1-se45)*(1-se46) + (1-p)*sp46
prod23[2] <- p*(1-se45)*se46 + (1-p)*(1-sp46)
prod23[3] <- p*se45*(1-se46)
prod23[4] <- p*se45*se46
prod24[1] <- p*(1-se47)*(1-se48) + (1-p)*sp48
prod24[2] <- p*(1-se47)*se48 + (1-p)*(1-sp48)
prod24[3] <- p*se47*(1-se48)
prod24[4] <- p*se47*se48
prod25[1] <- p*(1-se49)*(1-se50) + (1-p)*sp50
prod25[2] <- p*(1-se49)*se50 + (1-p)*(1-sp50)
prod25[3] <- p*se49*(1-se50)
prod25[4] <- p*se49*se50
prod26[1] <- p*(1-se51)*(1-se52) + (1-p)*sp52
prod26[2] <- p*(1-se51)*se52 + (1-p)*(1-sp52)
prod26[3] <- p*se51*(1-se52)
prod26[4] <- p*se51*se52
prod27[1] <- p*(1-se53)*(1-se54) + (1-p)*sp54
prod27[2] <- p*(1-se53)*se54 + (1-p)*(1-sp54)
prod27[3] <- p*se53*(1-se54)
prod27[4] <- p*se53*se54
prod28[1] <- p*(1-se55)*(1-se56) + (1-p)*sp56
prod28[2] <- p*(1-se55)*se56 + (1-p)*(1-sp56)
prod28[3] <- p*se55*(1-se56)
prod28[4] <- p*se55*se56
prod29[1] <- p*(1-se57)*(1-se58) + (1-p)*sp58
prod29[2] <- p*(1-se57)*se58 + (1-p)*(1-sp58)
prod29[3] <- p*se57*(1-se58)
prod29[4] <- p*se57*se58
prod30[1] <- p*(1-se59)*(1-se60) + (1-p)*sp60
prod30[2] <- p*(1-se59)*se60 + (1-p)*(1-sp60)
prod30[3] <- p*se59*(1-se60)
prod30[4] <- p*se59*se60
prod31[1] <- p*(1-se61)*(1-se62) + (1-p)*sp62
prod31[2] <- p*(1-se61)*se62 + (1-p)*(1-sp62)
prod31[3] <- p*se61*(1-se62)
prod31[4] <- p*se61*se62
prod32[1] <- p*(1-se63)*(1-se64) + (1-p)*sp64
prod32[2] <- p*(1-se63)*se64 + (1-p)*(1-sp64)
prod32[3] <- p*se63*(1-se64)
prod32[4] <- p*se63*se64
prod33[1] <- p*(1-se65)*(1-se66) + (1-p)*sp66
prod33[2] <- p*(1-se65)*se66 + (1-p)*(1-sp66)
prod33[3] <- p*se65*(1-se66)
prod33[4] <- p*se65*se66
prod34[1] <- p*(1-se67)*(1-se68) + (1-p)*sp68
```

```
prod34[2] <- p*(1-se67)*se68 + (1-p)*(1-sp68)
prod34[3] <- p*se67*(1-se68)
prod34[4] <- p*se67*se68
prod35[1] <- p*(1-se69)*(1-se70) + (1-p)*sp70
prod35[2] <- p*(1-se69)*se70 + (1-p)*(1-sp70)
prod35[3] <- p*se69*(1-se70)
prod35[4] <- p*se69*se70
prod36[1] <- p*(1-se71)*(1-se72) + (1-p)*sp72
prod36[2] <- p*(1-se71)*se72 + (1-p)*(1-sp72)
prod36[3] <- p*se71*(1-se72)
prod36[4] <- p*se71*se72
prod37[1] <- p*(1-se73)*(1-se74) + (1-p)*sp74
prod37[2] <- p*(1-se73)*se74 + (1-p)*(1-sp74)
prod37[3] <- p*se73*(1-se74)
prod37[4] <- p*se73*se74
prod38[1] <- p*(1-se75)*(1-se76) + (1-p)*sp76
prod38[2] <- p*(1-se75)*se76 + (1-p)*(1-sp76)
prod38[3] <- p*se75*(1-se76)
prod38[4] <- p*se75*se76
prod39[1] <- p*(1-se77)*(1-se78) + (1-p)*sp78
prod39[2] <- p*(1-se77)*se78 + (1-p)*(1-sp78)
prod39[3] <- p*se77*(1-se78)
prod39[4] <- p*se77*se78
prod40[1] <- p*(1-se79)*(1-se80) + (1-p)*sp80
prod40[2] <- p*(1-se79)*se80 + (1-p)*(1-sp80)
prod40[3] <- p*se79*(1-se80)
prod40[4] <- p*se79*se80

p ~ dbeta(1,1)
se1 ~dbeta(1,1)
se2 ~dbeta(1,1)
sp2 ~dbeta(1,1)
……
……
se80 ~dbeta(1,1)
sp80 ~dbeta(1,1)

}
```

```
list(n=58, rod1=c(2,26,0,30), rod2=c(3,25,0,30), rod3=c(6,22,0,30), rod4=c(6,22,1,29),
rod5=c(8,20,1,29), rod6=c(9,19,1,29), rod7=c(10,18,1,29), rod8=c(11,17,1,29),
rod9=c(13,15,3,27), rod10=c(14,14,3,27), rod11=c(14,14,5,25), rod12=c(15,13,6,24),
rod13=c(16,12,7,23), rod14=c(17,11,9,21), rod15=c(18,10,9,21), rod16=c(19,9,9,21),
rod17=c(20,8,9,21), rod18=c(20,8,10,20), rod19=c(20,8,12,18), rod20=c(20,8,14,16),
rod21=c(22,6,14,16), rod22=c(22,6,15,15), rod23=c(24,4,15,15), rod24=c(25,3,16,14),
rod25=c(26,2,16,14), rod26=c(27,1,16,14), rod27=c(27,1,17,13), rod28=c(28,0,18,12),
rod29=c(28,0,19,11), rod30=c(28,0,20,10), rod31=c(28,0,21,9), rod32=c(28,0,22,8),
rod33=c(28,0,23,7), rod34=c(28,0,24,6), rod35=c(28,0,25,5), rod36=c(28,0,26,4),
 rod37=c(28,0,27,3), rod38=c(28,0,28,2), rod39=c(28,0,29,1), rod40=c(28,0,30,0))
```

4.2. Comparison of non-parametric ROC analysis and Bayesian ROC analysis (non informative opinion) of ELISA results obtained with serum samples from Cameroonian pigs

	NON PARAMETRIC ROC		BAYESIAN ROC APPROACH ANALYSIS			
Cut-off- ELISA	**ANALYSIS**		**(non-informative opinion)**			
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	95 % CI	Specificity (%)	95 % CI
0.1	100	4	97	89 ; 100	12	2 ; 28
0.12	100	7	97	89 ; 100	16	4 ; 34
0.15	100	18	97	88 ; 100	28	11; 50
0.16	97	21	95	87 ; 100	28	12 ; 51
0.17	97	25	94	83 ; 99	36	17 ; 60
0.19	97	29	94	83 ; 99	40	20 ; 64
0.2	97	32	94	83 ; 99	44	24 ; 69
0.21	97	36	94	83 ; 99	48	27 ; 74
0.22	93	46	88	74 ; 96	55	33 ; 81
0.23	90	46	88	74 ; 96	59	36 ; 86
0.24	86	50	81	66 ; 92	58	35 ; 83
0.25	83	54	78	63 ; 90	61	38 ; 86
0.26	80	54	75	59 ; 88	64	42 ; 89
0.28	73	64	69	52 ; 83	67	44 ; 90
0.29	70	64	69	52 ; 83	77	48 ; 93
0.3	70	68	68	52 ; 83	75	52 ; 95
0.31	67	68	68	52 ; 83	78	57 ; 97
0.32	67	71	65	49 ; 80	78	56 ; 98
0.33	63	71	59	42 ; 75	77	57 ; 97
0.34	57	71	53	36 ; 69	77	56 ; 97
0.35	53	75	53	36 ; 69	82	62 ; 98
0.36	53	79	50	33 ; 66	81	62 ; 99
0.37	50	78	49	33 ; 66	88	71 ; 99
0.38	50	89	46	29 ; 63	90	75 ; 100
0.41	47	89	45	29 ; 62	93	79 ; 100
0.42	47	93	45	28 ; 62	95	83 ; 100
0.45	47	96	42	26 ; 59	95	83 ; 100
0.48	43	100	38	22 ; 56	96	87 ; 100
0.49	40	100	35	20 ; 52	96	87 ; 100
0.52	37	100	31	17 ; 49	96	86 ; 100
0.55	33	100	28	15 ; 45	96	87 ; 100
0.6	30	100	25	13 ; 41	96	87 ; 100
0.75	27	100	22	11 ; 38	96	86 ; 100
0.77	23	100	19	8 ; 35	96	86 ; 100
0.89	20	100	16	7 ; 31	96	86 ; 100
0.9	17	100	14	5 ; 27	97	86 ; 100
1.1	13	100	11	3 ; 23	96	86 ; 100
1.18	10	100	8	2 ; 19	96	85 ; 100
1.44	7	100	5	1 ; 15	96	85 ; 100
1.68	3	100	2	1 ; 10	96	85 ; 100