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(54) Title: EQUINE PARASITE DETECTION

(57) Abstract: The present invention provides a method of diagnosing a cyathostomin infection, said method comprising the step of identifying a level of anti- cyathostomin larval antigen antibodies in a sample, wherein a level of anti-cyathostomin larval antigen antibodies is indicative of a cyathostomin infection.

EQUINE PARASITE DETECTION

FIELD OF THE INVENTION

The present invention provides a means of detecting infections caused by parasitic nematodes belonging to the Cyathostominae group in Equine subjects and in particular in horses.

BACKGROUND OF THE INVENTION

Members of the Cyathostominae group of nematodes infect almost all grazing horses. Most horses have burdens to the order of tens of thousands of cyathostomins and usually do not exhibit clinical disease, however, in some animals, infection leads to a severe inflammatory enteropathy [15]. This disease occurs following accumulation of cyathostomin larvae that encyst and undergo inhibited development as early third larvae (EL3) in the large intestinal wall. Vast numbers of encysted larvae can accumulate and these can reactivate simultaneously to cause an inflammatory enteropathy known as larval cyathostominosis. The principal effect of this syndrome is weight loss, but horses can exhibit other signs including diarrhoea, colic, subcutaneous oedema and/or pyrexia [25]. Up to 50% of animals with larval cyathostominosis die as a result of the condition [15]. This disease most commonly occurs in younger horses, however horses have a lifelong susceptibility to infection and disease may occur at any age [15, 35]. Encysted larvae can persist for prolonged periods (up to two years in some cases) and it has been proposed that encystment is favoured by a variety of factors including; negative feedback from mature worms in the large intestinal lumen, a large larval challenge or a 'trickle' infection [29]. cyathostomin EL3 have limited susceptibility to several currently available anthelmintics [12, 19] and drug resistance is common, particularly with regard to benzimidazole and pyrantel compounds [17]. Moxidectin is now only drug available that has high efficacy against EL3, but for which resistance is not yet widespread. It is therefore important that the high efficacy of this anthelmintic be maintained for as long as possible.

To reduce the spread of anthelmintic resistance, it is important that only animals with moderate to high cyathostomin burdens are targeted strategically for treatment [32]. Targeted treatments can be undertaken on the basis of faecal egg counts however the latter have no value in estimating burdens of mucosal larvae. Indeed, horses with high mucosal burdens often have low or negative faecal egg counts [31] and there is no specific, non-invasive method to diagnose pre-patent cyathostomin infection. A

diagnostic test for mucosal larvae would allow veterinarians to identify horses that require larvicidal anthelmintic treatments. Recently, we identified two larval antigen complexes (observed to migrate at 20 and 25 kDa by 1-dimensional SDS PAGE) that have diagnostic potential [9-11]. Significant increases in serum IgG(T) specific to these antigen complexes were observed as early as 6 weeks post infection (PI) in experimentally-infected ponies [11]. Antigens present in both complexes appeared to be specific for mucosal larval cyathostomins, indicating their utility as markers of pre-patent infection [11]. When serum IgG(T) levels were compared amongst groups of naturally- and experimentally-infected horses, there was a strong significant correlation of anti-25kDa serum IgG(T) responses with total mucosal burden, particularly EL3 burden [10]. In naturally infected horses, IgG(T) responses to both larval complexes were significantly greater than those in uninfected individuals [10] and IgG(T) levels to both complexes were significantly higher in larval cyathostominosis clinical cases than in helminth-naïve ponies and parasite-negative horses from an abattoir [10]. These results indicate that an immunoassay based on antigens present in these complexes could ultimately be used to differentially diagnose larval cyathostominosis, or used to target horses with high mucosal burdens for treatment. The native mucosal larval preparations are extremely time-consuming to prepare and rely on a continuous source of infected mucosa. Therefore, it would be advantageous if genes encoding proteins present in these complexes were isolated and cloned and the associated proteins expressed in recombinant form.

SUMMARY OF THE INVENTION

The present invention is based upon the finding that parasitic nematodes belonging to the Cyathostominae group express proteins which can be used to diagnose, detect or identify incidences of cyathostomin infection in animals, particularly horses. Although cyathostomin infections are treatable, the range of effective drugs is rapidly diminishing and at present only moxidectin exhibits a high efficacy against the encysted cyathostomin parasite.

In order to ensure that the development of resistance to moxidectin can be delayed for as long as possible, it is essential that only animals with moderate to high cyathostomin burdens are targeted for treatment. However, the encysted larval stages of this parasite can remain undetected for months or even years eventually emerging from the intestinal wall to cause severe pathology (including symptoms of diarrhoea,

weight loss, colic, oedema and pyrexia); as such, it is often difficult to know whether or not a particular animal should be treated.

The inventors have identified a number of proteins that are expressed predominantly during the mucosal larval stages (i.e. the early third larval (EL3) and late third (LL3)/developing fourth (DL4) stages). These proteins are highly immunogenic and exhibit low cross-reactivity to proteins present in other helminth species.

Accordingly, a first aspect of this invention provides a method of diagnosing a cyathostomin infection, said method comprising the step of identifying a level of anti-cyathostomin larval antigen antibodies in a sample, wherein a level of anti-cyathostomin larval antigen antibodies is indicative of a cyathostomin infection.

Animals positively diagnosed as having a "cyathostomin infection" by the method provided by the first aspect of this invention may harbour high numbers of encysted cyathostomin in the gut mucosa, particularly the large intestinal wall, as such they may generate a significant immune response to cyathostomin antigens including any antigens produced by the EL3, LL3 and DL4 stages. Animals with infections of this type may otherwise be referred to as having high mucosal burdens. In other instances, positive diagnoses may indicate animals with larval cyathostominosis, an inflammatory enteropathy manifesting with symptoms of weight loss, diarrhoea, colic, subcutaneous oedema and/or pyrexia. Conditions of this type are often fatal if untreated.

In one embodiment, "a level" of anti-cyathostomin larval antigen antibodies may be evaluated relative to the "a level" of anti-cyathostomin larval antigen antibodies present in reference or control samples derived from healthy animals or animals not having high mucosal burdens of cyathostomin parasites or larval cyathostominosis. In this way levels and, in particular high levels, of anti-cyathostomin larval antigen antibodies, may easily be detected. Accordingly, the term "a level" may be taken to include levels of anti-cyathostomin larval antigen antibodies which are less or greater than levels of anti-cyathostomin larval antigen antibodies identified in reference or control samples.

It should be understood that in addition to providing methods in which levels of anti-cyathostomin larval antigen antibodies are detected in samples, the present invention might also be adapted to provide methods in which levels of cyathostomin larval antigens are detected in samples. Methods of this type, rather than "indirectly"

diagnosing cyathostomin infections via immune responses, may provide a more direct means of diagnosing cyathostomin infections. As above, "a level" of cyathostomin larval antigens may be taken to include levels of anti-cyathostomin larval antigen which are less or greater than levels of anti-cyathostomin larval antigen identified in reference or control samples.

The cyathostomin larval antigens described/mentioned herein may be derived from larval antigen complexes having a molecular weight of about 20 to about 25 kDa. An exemplary larval antigen is obtained from the parasite *cyathostomin pateratum* and comprises or consists of the following amino acid sequence (designated SEQ ID NO: 1):

SEQ ID NO: 1

MNKTLTFLTVVSAVALAQGVMDLFGEEGREEHRRHHRHSLLPPYLHNVSCEAKWEYF
KIVGNRSLTFAEKRKEISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSALPN
AVKKVNDLLDNENQTPRQLYVALRKLGRQNPALYRIVEYINVAVRLRSEEVDEQEQR
RRLSALPFGDHNDNLEEQDFGEQDFRYVYGFECARFLLQNGRMFGLNTDERY

The nucleic acid sequence encoding the protein provided by SEQ ID NO: 1 has also been determined and is given as SEQ ID NO: 2 below.

SEQ ID NO: 2

Atgaacaaaacgttaacatttctcacagtcgttagtgccgtagctctggcccaaggt
gtcatggacctttttggtgaagagggtcgtgaagaacatcgtcgtcaccatcgtcat
tcactttaccaccatatctccacaatgtgagctgtgaggctaaatgggagtacttc
aaaattgtggggaacaggagtttgacctttgctgagaaaagaaaggaaattagcgag
tgggcaaaaaaatacaatgttgtggatgaagttgcaagctacaatgcttacagggaa
aaactcaagcaggagcacagaaaaaacgttagcgaacttgtttctgctcttccaaac
gcagtgaagaaagtcaatgatcttctagacaatgaaaatcagactcctaggcaactt
tacgttgcccttagaaaacttggtagacaaaatccggcactttaccgtattgtcgag
tacattaatgtggctgtaagactaagaagtgaagaagtggatgagcaagaacaacga
agaaggctgtcagctctacctttggcgaccataacgataatttggaagagcaggac
ttcggtgaacaagactttcgctatgtctatggctttgagtgtgcaagatttctcctt
caaaatggaagaatgtttggacttaacacagatgaaagatat

One of skill in the art will appreciate that while SEQ ID NO: 1 represents the entire coding sequence of an exemplary cyathostomin larval antigen, after removal of the signal peptide the mature antigen may comprise 206 amino acids yielding a protein having a molecular weight of approximately 25.6 kDa.

In addition, the inventors have isolated homologous antigens from other cyathostomin spp., and the amino acid sequences of these are provided below as SEQ ID NOS: 3, 5 and 7 respectively. In addition, the nucleic acid sequences encoding each of the proteins encoded by SEQ ID NOS: 3, 5 and 7 have been designated SEQ ID NOS: 4, 6 and 8 respectively and each is detailed below.

SEQ ID NO: 3

HEELRRHHRHSLLPPYLHNVSCEAKWEYFKIVGNRSLTFAEKKGKSSEWAKKYNVVD
EVASYNAYREKLKQEHRKNVSELVSGLPGAVKKVNELLDNENQTPRQLYVALRKLGK
QNPVLYRVVEFVNLVVRFRREDSDEQEQREMLSTLPFSENNEEQDLGEQDFQYIYGF
ECARFIFQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 3 was isolated from *Cylicocyclus nassatus*.

SEQ ID NO: 4

Catgaagaacttcgtcgtcaccatcgtcattcacttttaccaccctatctccacaat
gtgagctgtgaagccaaatgggaatacttcaagattgtggggaacaggagcttgact
tttgctgaaaagaagggaaaaagtagcgagtgggcaaaaaaatacaatgttgtggat
gaagttgcaagttacaatgcctatagagaaaaacttaagcaggagcacaggaaaaac
gttagcgaacttgtttctggtcttcccggtgctgtgaagaaagtaaacgaactcttg
gataatgagaatcagactcctaggcaactttacgttgctctaagaaagcttggtaaa
caaaatccagtactctaccgtgttgtcgagtttgtcaatttggttgtgagatttaga
cgtgaagattcggatgagcaagaacaacgagaaatgctgtcaactttacctttcagc
gaaaataatgaagagcaggaccttggtgaacaagacttccagtacatctatggtttt
gaatgtgcaagattcatctttcaaaatgggagaatgtttggactcaacacggataga
agatat

SEQ ID NO: 5

SCVAKWEYFKIVINRSLTFAQRKEEISKWAKKYKVEDEVASYNAYREKLKQQHRKNV
SELVSNLPGAVERVNKLLDNENQTPKQLYLALRELGKQNPALYHVVEYVNVVVRLKR
EELDQQDQRRALSGSLFGENNDNLEEQDFGEEDFRYVYGFECARFILQNGRMFGLNM
DRNY

The antigen encoded by SEQ ID NO: 5 was isolated from *Coronocyclus coronatus*.

SEQ ID NO: 6

Agctgtgtggctaagtgggagtacttcaagatcgtgatgaacaggagtctgacgttt
gctcaaagaaaggaagaaattagcaagtgggcgaaaaaatacaaagttgaggatgaa
gttgcaagctacaatgcttatagagaaaaactcaagcagcagcacaggaaaaacgtt
agcgaacttgtttctagtcttcccggtgcaatggaaagagtgaacaaacttttggac
aatgaaaaccagacccctaagcaactttaccttgccctacgagaacttggcaaacaa
aatccggcactttaccatgttgtcgagtatgtcaatgtggttgtgagacttaaacga
gaagaattggatgaacaagatcaatgaagagcgctgtcgggttcacttttggcgag
aataacgacaatctagaagagcaggactttggtgaagaagactttcgctatgtctat
gggtttgaatgtgcaagattcatccttcaaaatggaagaatgtttggtctaaacatg
gataggaattat

SEQ ID NO: 7

GEEDREEHRRHHRHSLLPPYLHNVSCVAKWEYFRIVGNRSLTFAEKKKEISEWAKKY
NVLDEVASYNAYREKLKQEHRKNVSELVSDLPKAVKKVNDLLDNENQTPRQLYVALR
ELGRQNPTLYRIVEYINVAVRRRSEELDEQEQGRRLSALPFGDNNDNLEEQDFGEQD
FRYVYGFECARFLLQNGRMFGLNTDERD

The antigen encoded by SEQ ID NO: 7 was isolated from *Cyathostomum catinatum*.

SEQ ID NO: 8

Gaggatcgtgaagaacatcgccgtcaccatcgtcattcactcttgccaccatatctc
cacaacgtgagctgtgtggccaaatgggaatactttagaattgtggggaacaggagt
ttaacgtttgctgagaaaaagaaagaaattagcgagtgggcaaaaaaatacaatgtt

ctggatgaagtagcaagctacaatgcttatagggaaaaactcaagcaggagcacaga
aaaaacgttagcgaacttgtttctgatcttcccaaggcagtaaagaaagtcaacgat
cttctagacaatgaaaatcagactcctaggcaactttatgttgcccttagagagctt
ggtagacaaaatccgacactttaccgtattgtcgagtacatcaatgtggctgtaagg
cgaagaagtgaagaactggatgagcaagaacaaggaagaaggctgtcagctttacct
ttcggcgacaacaacgataatttggaagagcaggacttcggtgaacaagactttcgc
tatgtctacggctttgagtgtgcaagatttctccttcaaaatggaagaatgttcgga
ctcaacacagatgaaagagat

SEQ ID NOS 9-22 have been translated into amino acid sequences by removing the non-coding regions (introns) identified by comparison with the coding sequence of Gala-1.

The antigen encoded by SEQ ID NO: 9 was isolated from *Cylicocyclus ashworthi.*

SEQ ID NO: 9

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTAGTTCTGGCCCAAGGT
GTCATGGACCTTTTTTGGTGAAGAGGGTCGTGAAGAACATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAACGTGAGCTGTGTGGCTAAATGGGAGTACTTC
AAAATTGTAGGGAACAGGAGTTTAACGTTTGCTGAGAAAAAAGAAGAAATTAGCCAG
TGGGCAAAAAAATACAATGTTGTGGTAAGCTTTTCTGAATTAATGTAAATACACTCG
CATGCTGGCCTTTTTAGGATGAAGTTGCAAGCTACAATGCTTACAGGGAGAAACTCA
AGCAGGAGCACAGAAAAAACGTTAGCGAACTTGTTTCTGCTCTTCCAAACGCAGTAA
AGAAAGTCAACAATCTTCTAGACAATGAAAATCAGACTCTTAGGCAACTTTACGTTG
CCCTTAGAGAACTTGGTAGACAAAATCCGGCAGTAAGTAGAAAGAGCTGCACTCCTG
GGCTTAATAAAACAAATTATTTAAGCTTTACCGTATTGTCGAGTACATCAATGTGGC
TGTAAGACGAAGAAGTGAAGGACTGGATGAGCAAGAACAACGAAGAAAGCTATCAGC
TTTACCTTTCGGCGACAACAACGATAATATGGAAGAGCAGGACTTCGGTGAACAAGA
CTTTCGCTATGTCTACGGCTTTGAGTGTGCAAGATTTCTCCTTCAAAATGGAAGAAT
GTTTGGGCTCAACACAGATGAAAGAGATTAGCAAAGAATCAATTGTAGTTCAAAGCG
GTAGAGTTTGAGCTGCAAACTCAGCATGCCATCATCACCTCCT

SEQ ID NO: 9A translated

MNKTLTFLTVVSAVVLAQGVMDLFGEEGREEHRRHHRHSLLPPYLHNVSCVAKWEYF
KIVGNRSLTFAEKKEEISQWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSALPN
AVKKVNNLLDNENQTLRQLYVALRELGRQNPALYRIVEYINVAVRRRSEGLDEQEQR
RKLSALPFGDNNDNMEEQDFGEQDFRYVYGFECARFLLQNGRMFGLNTDERD

The antigen encoded by SEQ ID NO: 10 was isolated from *Cyathostomum catinatum*.

SEQ ID NO: 10

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTAGTCCTGGCT
CAAGGTGTCATGGACCTTTTTGGTGAAGAAGGCCGTGAAGAACATCGCCG
TCACCGTCGTCATTCACTCTTGCCACCATATCTCCACAACGTGAGCTGTGT
GGCTAAATGGGAATACTTCAGAATTGTGGGGAACAGGAGTTTGACGTTTG
CTGAGAAAAAGGAAGAGATTAGCGAGTGGGCAAAAAAGTACAATGTTGT
GGTAAGCTTTTCTGAATTGATGTAAATACACTCGCATGCTGGCCTTTTTAG
GATGAAGTTGCAAGCTACAATGCTTACAGGGAAAAACTCAAGCAGGAGC
ACAGAAAAAACGTTAGCGAACTTGTTTCTGCTCTTCCAAACGCAGTAAAG
AAAGTCAACGATCTTCTAGACAATGAAAATCAGACTCCTAGGCAACTTTA
CGTTGCCCTTAGAGAACTTGGTAGACAAAATCCGGCAGTAAGTCGAAAGA
GCTGCACTCTTGGGCATAAGTAAAAAAAAGTATTTTAGCTTTACCGTATTG
TGGAGTACATCAATGTGGCTGTAAGACTAAGAAGTGAAGAAGTGGATGA
GCAAGAACAACGAAGAAGGCTATCAGCTTTACCTTTTGGTGACCATAACG
ATAATATGGAAGAGCAGGACTTTGGTGATCAAGACTTTCGCTATGTCTAC
GGCTTTGAGTGTGCAAGATTTCTCCTTCAAAATGGAAGAATGTTTGGACTT
AACACAGATGAAAGATATTAGTAAAAATTAACTGTAGCTCAAAGCGGTAG
AGTTTGAGCTGCAAACTCAGCATGCCATCATCACCTCCT

SEQ ID N0 10A translated

MNKTLTFLTVVSAVVLAQGVMDLFGEEGREEHRRHRRHSLLPPYLHNVSCVAKWEYF
RIVGNRSLTFAEKKEEISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSALPN
AVKKVNDLLDNENQTPRQLYVALRELGRQNPALYRIVEYINVAVRLRSEEVDEQEQR
RRLSALPFGDHNDNMEEQDFGDQDFRYVYGFECARFLLQNGRMFGLNTDERY

The antigen encoded by SEQ ID NO: 11 was isolated from *Cylicostephanus goldi* .

SEQ ID NO: 11

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTAGTCCTGGCTCAAGGT
GTCGTGGACCTTTTTGGTGAAGAGGGTCGTGAAGAACATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAACGTCAGCTGTGTGGCTAAATGGGAATACTTC
AAAATTGTGGGGAATAGGAGTTTGACATTTGCTGAGAAAAAGAAAGAAATTAGCGAG
TGGGCTAAAAAATACAATGTAGTGGTAAGCTTTTTTGACTTGATGTAAATGCACTCG
TATGCCGGCCCTTTTAGGATGAAGTTGCAAGGTACAATGCTTATAGAGAAAAACTTA
AGCAGGAACACAGGAAAAACGTCAGCGAACTTGTTTCTGATCTTCCCAACGCAGTAA
AGAAAGTGAATGATCTCCTGGACAATGAGAATCAAACTCCTAGGCAACTTTACATTG
CCCTCAGAGAACTTGGTAGACAAAATCCAGAAGTAAGTTGAAAGTGCTGCAATTTTA
GGCTTAGATAAAACAGTTGTTTAAGCTTTACCGTGTTGTCGAGTTTATCAATGTGGC
TGTAAGAATAAGACGTGAAGATTTGGATGAGCAAGAACAACGAACAAGGCTGTCAAC
TTTACCTTTTGGCGACAACAACGACAATTTCGAAGAGCAAGACTTCGGTGAACAAGA
CTTTCGCTATGTCTATGGCTTTGAGTGTGCAAGATTTCTCCTTCAAAATGGAAGAAT
GTTTGGACTTAACACGGATAGAAGATAC

SEQ ID NO 11A translated

MNKTLTFLTVVSAVVLAQGVVDLFGEEGREEHRRHHRHSLLPPYLHNVSCVAKWEYF
KIVGNRSLTFAEKKKEISEWAKKYNVVDEVARYNAYREKLKQEHRKNVSELVSDLPN
AVKKVNDLLDNENQTPRQLYIALRELGRQNPELYRVVEFINVAVRIRREDLDEQEQR
TRLSTLPFGDNNDNFEEQDFGEQDFRYVYGFECARFLLQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 12 was isolated from *Cylicostephanus goldi*

SEQ ID NO: 12

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTAGTCCTGGCCCAAGGT
GTCATGGACCTTCTTGATGAAGAGGCTCGTGGAGAGCATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAACGTGAGCTGTGTGGCTAAATGGGAATACTTC
AAAATTGTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGAAAGAAATTAGCGAG
TGGGCAAAAAAATACAACGTTGTGGTAAGCTTTTGTGACTCGATGTAGATACCCCAG

ATATTCTAGATACCCATGCTGGCCTTTTTAGGATGAAGTTGCAAGCTACAATGCTTA
TAGAGAAAAACTCAAGCAGGAACACAGGAAAAACGTTAGCGAACTTGTATCTGATCT
TCCCAATGCAGTGAAGAAAGTGAATGATCTCCTGGACAATGAGAATCAAACTCCTAG
GCAACTTTACGTTGCCCTCAGAGAACTTGGTAGACAAAATCCAGCAGTAAGTTGAAA
GTGCTGCAATTTCAGGCTTAGATAAAACAGTTGTTTAAGCTTTACCGTGTTGTCGAG
CTCATCAATGTGGCTGTAAGATTAAGACGTGAAGATTTGGATGAGCAAGAACAACGA
ACAAGGCTGTCAACCTTACCTTTTGGCGACAACAACAACAATTTCGATGAGCAGGAC
TTCGGTGAACAAGACTTTCGCTATGTCTATGGCTTTGAGTGTGCAAGATTTCTCCTT
CAAAATGGAAGAATGTTTGGACTTAACACGGATAGAAGATACTAGTAAGAGTCAACT
GTAGCTCAAAGTGGTTCGAGCTACGAACAGCATGCCATCATCACCTCCT

SEQ ID NO 12A translated

MNKTLTFLTVVSAVVLAQGVMDLLDEEARGEHRRHHRHSLLPPYLHNVSCVAKWEYF
KIVGNRSLTFAEKKKEISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSDLPN
AVKKVNDLLDNENQTPRQLYVALRELGRQNPALYRVVELINVAVRLRREDLDEQEQR
TRLSTLPFGDNNNNFDEQDFGEQDFRYVYGFECARFLLQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 13 was isolated from *Cylicostephanus goldi*

SEQ ID NO: 13

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTTGTCCTGGCGCAAGGT
GTCATGGCCCTATTTGGTGAAGAGAGTCGTGAAGAACACCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAACGTGAGCTGTGTGGCTAAATGGGAGTACTTC
AAAATTGTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGAAAGAAATCAGCGAG
TGGGCTAAAAAATACAATGTTGTGGTAAGCTTTTTTGACTTGATGTAAATGCACTCG
CATGCCGGCCTTTATAGGATGAAGTTGCAAGCTACAATGCTTATAGAGAAAAACTCA
AGCAGGAACACAGGAAAAACGTTAGCGAACTTGTTTCTGATCTTCCCAACGCAGTAA
AGAAAGTCAGCGATCTTTTGGACAACGAAAATCAGACTTCTAGGCAACTTTATGTTG
CACTCAGAGAACTTGGTAGACAAAATCCGGCAGTAAGTTGAAGAGGCTCCAATTTTG
GGCTCAAGCAAAAATAATTATTTTAGCTATACCGTGTCGTCGAGTATATCAATGTGG
CTGTGAGATTAAGACGAAAAGAACAGGATGAACAAGAACGACAAGGAACGCTGTCAG
CTCTACCTTTTGGCGAGAATAACGACAATTTGGAAGAGCAGGACTTTGGTGAACAAG
ACTTTCGCTATGTCTATGGCTTTGAGTGTGCAAGATTTCTCCTTCAAAATGGAAGAA

TGTTTGGACTCAACACGGATAGAAGATACCAGTAAGAGTCAACTGTAGCTCAAAGTG
GGTTTGAGCTACGAACAGCATGCCATCATCACCTCCT

SEQ ID NO 13A translated

MNKTLTFLTVVSAVVLAQGVMALFGEESREEHRRHHRHSLLPPYLHNVSCVAKWEYF
KIVGNRSLTFAEKKKEISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSDLPN
AVKKVSDLLDNENQTSRQLYVALRELGRQNPAVYRVVEYINVAVRLRRKEQDEQERQ
GTLSALPFGENNDNLEEQDFGEQDFRYVYGFECARFLLQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 14 was isolated from *Cylicostephanus longibursatus*

SEQ ID NO: 14

ATGAACAAAACGTTAACATTTCTCACCGTCGTCTATGCCGTAGTCCTGGCCCAAGGT
GTCATGGACCTTTTTGGTGAAGAGGGTCGTGAAGAACATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAATGTGAGCTGTGTGGCTAAATGGGAATACTTC
AAAATTGTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGGAAGAAATTAGCAAG
TGGGCAAAAAAATACAATGTTGTGGTACGCTTTTGTAACCCCGTATAATATACTCTC
GCATACTGGCCGTTTCAGGATGAAGTTGCAAGCTACAGTGCTTGCAGGGAAAAGCTT
AAGCAGGAACACAGGAAAAACGTTAGCGAAATTGTTTCTAATCTTCCCAATGCAGTG
AAGAAAGTAAACGATCTTTTGGACAATGAAAATCAGACCCCCAGGCAACTTTACGTT
GCCTTCAGAAAACTTGGTAAACAAAATCCGGCAGTAAGTTGAAAGAGCTGCAATTTT
GGGTTTGAGGAGAAAAAACTATTTTAGCTTTATCGTGTTGTCGAGTATATCAATGTG
CTTGTGAGACTAAGACGTGAAGAATTTGATGAAGATCAGCGAAGATCGCTGTCAGCT
TTACCTTTTGGCGACAATAACGACGATTTGGAAGAGCAGGACTTTGGTGAACAGGAC
TTTCGCTATATCTATGGCTTTGAGTGTGCAAGATTTATCCTTCAAAATGGAAGAATG
TTCGGACTCAACACGGATAGAAGATATTAGTAAGAGTCAACTGTAGCTCGAGGGTTT
GAGCTACGAACTGCATGCCATCATCACCTCCT

SEQ ID NO 14A translated

MNKTLTFLTVVYAVVLAQGVMDLFGEEGREEHRRHHRHSLLPPYLHNVSCVAKWEYF
KIVGNRSLTFAEKKEEISKWAKKYNVVDEVASYSACREKLKQEHRKNVSEIVSNLPN
AVKKVNDLLDNENQTPRQLYVAFRKLGKQNPALYRVVEYINVLVRLRREEFDEDQRR
SLSALPFGDNNDDLEEQDFGEQDFRYIYGFECARFILQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 15 was isolated from *Cylicocyclus insigne.*

SEQ ID NO: 15

ATGAACAAAACGTTAACATTTCTCACCGTCGTCTGTGCCGTAGTCCTGGCCCAAGGT
GTCATGGACCTTTTTGGTGAAGAAGGTCGTGAAGAACATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAATGTGAGCTGTGTGGCTAAATGGGAATACTTC
AAAATTCTGGGGAACAGAAGTTTGACGTTTGCTGAGAAAAAGGAAAAAATCAGCGAG
TGGGCAAAAAAGTACAATGTTGTGGTACGCTTTTGTAACTCCGTATAATATACCCTC
GCATGCTGGCCGTTTCAGGATGAAGTTGCAAGCTACAATGCTTGCAGGGAAAAGCTT
AAGCAGGAACACAGGAAAAACGTTAGCGAAATTGTTTCTAATCTTCCCAATGCAGTA
AAGAAAGTAAACGATCTTTTGGACAATGAAAATCAGACTCCCAGGCAACTTTACGTT
GCCCTCAGAAAACTCGGTAAACAAAATCCGCCAGTAAGTTGAAAGACTGCAACTTTG
GGTTTAAGGGAAAAAAACTATTTTAGCTTTACCGCGTTGTCGAGTATATCAATGTGG
TTGTGAGACTAAGACGTGAAGAATCTGATGAAGAACAACGAAGAACGCTGTCAGCTT
TACCTTTTGGCGACAATAACGACAACTTGGAAGAGCAAGACTTTGGTGAAGAAGACT
TTCGCTATATTTATGGCTTTGAGTGTGCAAGATTTATCCTTCAAAATGGGAGAATGT
TCGGACTCAACACGGATAGAAGATATCAGTAAGAGTCAACTGTAGCTTAAAAGTTTG
AGCTACGAACAGCATGCCATCATCACCTCCT

SEQ ID NO. 15A translated

MNKTLTFLTVVCAVVLAQGVMDLFGEEGREEHRRHHRHSLLPPYLHNVSCVAKWEYF
KILGNRSLTFAEKKEKISEWAKKYNVVDEVASYNACREKLKQEHRKNVSEIVSNLPN
AVKKVNDLLDNENQTPRQLYVALRKLGKQNPPLYRVVEYINVVVRLRREESDEEQRR
TLSALPFGDNNDNLEEQDFGEEDFRYIYGFECARFILQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 16 was isolated from *Cylicostephanus longibursatus.*

SEQ ID NO: 16

ATGAACAAAACGTTAACATTTCTCACCGTCGTCTATGCCGTAGTCCTGGCCCAAGGT
GTCATGGACCTTTTTGGTGAAGAGGGTCTTGAAGAACATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAATGTGAGCTGTGTGGCTAAATGGGAATACTTC
AAAATTCTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGGAAAAAATCAGCGAG

TGGGCAAAAAAGTACAATGTTGTGGTACGCTTTTGTAACTCAGTATAATATATCCTC
GCATACTGGCCGTTTCAGGATGAAGTTGCAAGCTACAATGCTTGCAGGGAAAAGCTT
AAGCAGGAACACAGGAAAAACGTTAGCGAAATTGTTTCTAATCTTCCCAATGCAGTG
AAGAAAGTAAACGATCTTTTGGACAATGAAAATCAGACCCCCAGGCAACTTTACGTT
GCCCTCAGAAAACTTGGTAAACAAAATCCGGCAGTAAGTTGAAAGAGCTGCAATTTT
GGGTTTGAGGAAAAAAAACTATTTTAGCTTTATCGTGTTGTCGAGTATATCAATGTG
CTTGTGAGACTAAGACGTGAAGAATTTGATGAAGATCAGCGAAGATCGCTGTCAGCT
TTACCTTTTGGCGACAATAACGACGATTTGGAAGAGCAGGACTTTGGTGAACAGGAC
TTTCGCTATATCTATGGCTTTGAGTGTGCAAGATTTATCCTTCAAAATGGAAGAATG
TTCGGACTCAACACGGATAGAAGATATTAGTAAGAGTCAACTGTAGCTCAAGGGTTT
GAGCTACGAACTGCATGCCATCATCACCTCCT

SEQ ID NO 16A translated

MNKTLTFLTVVYAVVLAQGVMDLFGEEGLEEHRRHHRHSLLPPYLHNVSCVAKWEYF
KILGNRSLTFAEKKEKISEWAKKYNVVDEVASYNACREKLKQEHRKNVSEIVSNLPN
AVKKVNDLLDNENQTPRQLYVALRKLGKQNPALYRVVEYINVLVRLRREEFDEDQRR
SLSALPFGDNNDDLEEQDFGEQDFRYIYGFECARFILQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 17 was isolated from *Cylicocyclus nassatus*.

SEQ ID NO: 17

ATGAACAAAACGTTAACATTTCTCATCGTCGTTAGTGCCGTAGTCCTGACCCAAAGT
GTTATGGACTTTTTCGATGAAGACGGTCGTGAAGAACATCGCCGTCATCATCGTCAT
TCCCTTTTACCACCGTATCTCCACAATATGAGCTGCGTGGCCAAATGGGAATACTTC
GAGATTGTGGGGGACAGGAGTCTGACGTTTGCTGAAAAGAAGGAAAAAATCGGCGAG
TGGGCTAAAAAATACAATGTTGTGGTAAGATTTTGTAACTCTATGTAAAGATACCCC
CGTACGTCGCCCTGTTTAGGATGAAGTTGCAAGCTACAATGCTTATAGAGAAAAACT
AAAGCAGGAGCACAGGAAAAACGTTAGCGAGCTTGTCTCTGGTCTTCCCAATGCTGT
GAAGAAAATAAACGAACTTTTAGACAATGAAAATCAGACTGTTAGGCAACTTTATGT
TGCTTTAAGAGAACTTGGTAAACAAAATCCAGCAGTAAGTTAAAAGAAGTGCAATTT
TGGGCTTAACTAATGAGACAATTTTAGCTCTACCGTGTTGTCGAGTATATCAATGTG
GTTGTGAGACTTAGACGTGAAGATTTGGATGAGCAGGAACAACAGAGAACGCTGTCA
ACCCCACCTTTCGGCGAGAATAACGAAGAGCAAGACTTTGGTGAACAAGACTTTCAC

TATATCTATGGTTTTGAGTGTGCCAGATTCATCCTTCAAAATGGAAGAATGTTTGGA
CTTAACACGGATAGAAGATATTAGTAAGAGTTAACTGCAGCTCAATGTGATAGAGAT
TGAGCCACAACCCAACATGCCATCATCACCTCCT

SEQ ID NO. 17A translated
MNKTLTFLIVVSAVVLTQSVMDFFDEDGREEHRRHHRHSLLPPYLHNMSCVAKWEYF
EIVGDRSLTFAEKKEKIGEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSGLPN
AVKKINELLDNENQTVRQLYVALRELGKQNPALYRVVEYINVVVRLRREDLDEQEQQ
RTLSTPPFGENNEEQDFGEQDFHYIYGFECARFILQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 18 was isolated from *Cylicocyclus nassatus*.

SEQ ID NO: 18

ATGAACAAAACGTTAACATTTCTCATCGTCGTTAGTGCCATAGTCCTGGCCCAAAGT
GTTATGGACTTTTTCGATGAAGAAGGTCGTGAGGGACATCGCCGTCATCATCGTCAT
TCACTTTTACCACCATATCTCCACAATATGAGCTGCGTGGCCAAATGGGAATACTTC
GAGATTGTGGGGGACAGGAGTCTGACGTTTGCTGAAAAGAAGGAAAAAATCGGCGAG
TGGGCTAAAAAATACAATGTTGTGGTAAGATTTTGTAACTCCATGTTAGGATACCTC
CGCACGTCGCCCTGTTTAGGATGAAGTTGCAAGCTACAATGCTTATAGAGAAAAACT
AAAGCAGGAGCACAGGAAAAACGTTAGCGAGCTTGTCTCTGGTCTTCCCAATGCTGT
GAAGAAAGTAAACGAACTTTTAGACAATGAAAATCAGACTGTTAGGCAACTTTATGT
TGCTTTAAGAGAACTTGGTAAACAAAATCCAGCAGTAAGTTAAAAGAAGTACAATTT
TGAGCTCAACTAATGAGACAATTTTAGCTCTACCGTGTTGTCGAGTATATCAATGTG
GTTGTGAGACTTAGACGTGAAGATTCGGATGAGCAGGAACAACGAAGAACTCTGTCA
ACCTCACCTTTCGGCGAGAATAACGAAGAGCAAGATTTTGGTGAACAAGATTTTCAC
TATATCTATGGTTTTGAGTGTGCAAGATTCATCCTTCAAAATGGAAGAATGTTTGGA
CTCAATACGGATAGAAGATAT

SEQ ID NO 18A translated
MNKTLTFLIVVSAIVLAQSVMDFFDEEGREGHRRHHRHSLLPPYLHNMSCVAKWEYF
EIVGDRSLTFAEKKEKIGEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSGLPN
AVKKVNELLDNENQTVRQLYVALRELGKQNPALYRVVEYINVVVRLRREDSDEQEQR
RTLSTSPFGENNEEQDFGEQDFHYIYGFECARFILQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 19 was isolated from *Cylicocyclus nassatus*.

SEQ ID NO: 19

ATGAACAAAACGTTAACATTTCTCATCGCCGTTAGTGCCATAGTCCTGGCCCAAAGT
ATGGACTTTTTCGATGAAGACGGTCGTGAAGAACATCGCCGTCATCATCGTCATTCA
CTTTTACCACCATATCTCCACAATATGAGCTGCGCGGCCAAATGGGAATACTTCGAG
ATTGTAGGGGACAGGAGTCTGACGTTTGCTGAAAAGAAGGAAAAAATCGGCGAGTGG
GCTAAAAAATACAATGTTGTGGTAAGATTTTGTAACTCCATGTAAAGATACCCCTCC
ATGTCGTCCCGTTTAGGATGAAGTTGCAAGCTACAATGCTTGCAGAGAAAAACTGAA
GCAAGAGCACAGGAAAAACGTCAGCGAGCTTGTCTCTGGTCTTCCCAATGCTGTGAA
GAAAGTAAACGAACTTTTAGACAATGAAAATCAGACTGTTAGGCAACTTTATGTTGC
TTTAAGAGAACTTGGTAAACAAAATCCAGCAGTAAGTTGAAAGAAGTGCATTTTGGG
CTTAACTAACGAGACAATTTTAGCTCTACCGTGTTGTCGAGTATATCAATGTGGCTG
TGAGACTTAGACGTGAAGATTCGGATGAGCAGGAAAAACGAAGAACGCTGTCAACCT
CACCTTTCGGCGAGAATAACGAAGAGCAGGACCTTGGTGAACAAGATTTTCACTATA
TCTATGGCTTTGAGTGTGCAAGATTCATCCTTCAAAATGGAAGAATGTTTGGACTTA
ACACGGATAGAAGATATTAGTAAAATTTGACTGCAGCTCAAAGTGGTAGAGATTGAG
CTACCAACCCAACATGCCATCATCACCTCCT

SEQ ID NO. 19A translated

MNKTLTFLIAVSAIVLAQSMDFFDEDGREEHRRHHRHSLLPPYLHNMSCAAKWEYFE
IVGDRSLTFAEKKEKIGEWAKKYNVVDEVASYNACREKLKQEHRKNVSELVSGLPNA
VKKVNELLDNENQTVRQLYVALRELGKQNPALYRVVEYINVAVRLRREDSDEQEKRR
TLSTSPFGENNEEQDLGEQDFHYIYGFECARFILQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 20 was isolated from *Cyathostomum pateratum*.

SEQ ID NO: 20

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTAGTTCTGGCCCAAGGT
GTCATGGACCTTTTTGGTGAAGAGGGTCGTGAAGAACATCGTCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAATGTGAGCTGTGAGGCTAAATGGGAGTACTTC
AAAATTGTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGGAGAAAATTAGCGAG

TGGGCAAAAAAATACAATGTTGTGGTAAGCTTTTTTGAATTGATGTAAATTCACTCG
CATGCTGGCCTTTTTAGGATGAAGTTGCAAGCTACAATGCTTACAGGGAAAAACTCA
AGCAGGAGCACAGAAAAAACGTTAGCGAACTTGTTTCTGCTCTTCCAAACGCAGTAA
AGAAAGTCAACGATCTTCTAGACAATGAAAATCAGACTCTTAGGCAACTTTACGTTG
CCCTTAGAAAACTTGGTAGACAAAATCCGGCAGTAAGTCGAAAGAGCTGCGTCCTTG
GACTTAAGCGGAAAAATTATTTCAGCTTTACCGTATTGTCGAGTACATTAATGTGGC
TGTAAGACTAAGAAGTGAAGAAGTGGATGAGCAAGAACAACGAAGAAGGCTGTCAGC
TCTACCTTTTGGCGACCATAACGATAATTTGGAAGAGCAGGACTTCGGTGAACAAGA
CTTTCGCTATGTCTATGGCTTTGAGTGTGCAAGATTTCTCCTTCAAAATGGAAGAAT
GTTCGGACTCAACACGGATGGAAGATATTAGTAAGAAACAAGTGTAGCTCAAAGTGG
TAGAGTTTGAGCTACGAACTCAACATGCCATCATCACCTCCT

SEQ ID NO. 20A translated

MNKTLTFLTVVSAVVLAQGVMDLFGEEGREEHRRHHRHSLLPPYLHNVSCEAKWEYF
KIVGNRSLTFAEKKEKISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSALPN
AVKKVNDLLDNENQTLRQLYVALRKLGRQNPALYRIVEYINVAVRLRSEEVDEQEQR
RRLSALPFGDHNDNLEEQDFGEQDFRYVYGFECARFLLQNGRMFGLNTDGRY

The following sequences (SEQ ID NOS: 21 and 22) represent Cyathostomin GALA sequences obtained from cDNA clones.

The antigen encoded by SEQ ID NO: 21 was isolated from *Cylicostephanus goldi.*

SEQ ID NO: 21

ATGAACAAAACGTTAACATTTCTCACAGTCGTTAGTGCCGTTGTCCTGGCCCAAGGT
GTCATGGCCCTATTTGGTGAAGAGAGTCGTGAAGAACACCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAACGTGAGCTGTGTGGCTAAATGGGAGTACTTC
AAAATTGTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGAAAGAAATCAGCGAG
TGGGCTAAAAAATACAATGTTGTGGATGAAGTTGCAAGCTACAATGCTTATAGAGAA
AAACTCAAGCAGGAACACAGGAAAAACGTTAGCGAACTTGTTTCTGATCTTCCCAAC
GCAGTAAAGAAAGTCAACGATCTTTTGGACAACGAAAATCAGACTTCTAGGCAACTT
TATGTTGCACTCAGAGAACTTGGTAGACAAAATCCGGCACTATACCGTGTCGTCGAG
TATATCAATGTGGCTGTGAGATTAAGACGAAAAGAACAGGATGAACAAGAACGACAA
GGAACGCTGTCAGCTCTACCTTTTGGCGAGAATAACGACAATTTGGAAGAGCAGGAC

TTTGGTGAACAAGACTTTCGCTATGTCTATGGCTTTGAGTGTGCAAGATTTCTCCTT
CAAAATGGAAGAATGTTTGGACTCAACACGGATAGAAGATACCAGTAAGAGTCAACT
GTAGCTCAAAGTGGGTTTGAGCTACGAACAGCATGCCATCATCACCTCCT

SEQ ID NO. 21 translated
MNKTLTFLTVVSAVVLAQGVMALFGEESREEHRRHHRHSLLPPYLHNVSCV
AKWEYFKIVGNRSLTFAEKKKEISEWAKKYNVVDEVASYNAYREKLKQEHR
KNVSELVSDLPNAVKKVNDLLDNENQTSRQLYVALRELGRQNPALYRVVEY
INVAVRLRRKEQDEQERQGTLSALPFGENNDNLEEQDFGEQDFRYVYGFECA
RFLLQNGRMFGLNTDRRY

The antigen encoded by SEQ ID NO: 22 was isolated from *Cylicostephanus longibursatus.*

SEQ ID NO: 22

C.lon91-GALA
ATGAACAAAACGTTAACATTTCTCACCGTCGTCTATGCCGTAGTCCTGGCCCAAGGT
GTCATGGACCTTTTTGGTGAAGAGGGTCGTGAAGAACATCGCCGTCACCATCGTCAT
TCACTCTTACCACCATATCTCCACAATGTGAGCTGTGTGGCTAAATGGGAATACTTC
AAAATTCTGGGGAACAGGAGTTTGACGTTTGCTGAGAAAAAGGAAAAAATCAGCGAG
TGGGCAAAGAAGTACAATGTTGTGGATGAAGTTGCAAGCTATAATGCTTGCAGGGAA
AAGCTTAAGCAGGAACACAGGAAAAACGTTAGCGAAATTGTTTCTAATCTTCCCAAT
GCAGTGAAGAAAGTAAACGATCTTTTGGACAATGAAAATCAGACCCCCAGGCAACTT
TACGTTGCCCTCAGAAAACTTGGTAAACAAAATCCGGCACTTTATCGTGTTGTCGAG
TATATCAATGTGCTTGTGAGACTAAGACGTGAAGAATTTGATGAAGATCAACGAAGA
TCGCTGTCAGCTTTACCTTTTGGCGACAATAACGACGATTTGGAAGAGCAGGACTTT
GGTGAACAGGACTTTCGCTATATCTATGGCTTTGAGTGTGCAAGATTTATCCTTCAA
AATGGAAGAATGTTCGGAATCAACACGGATAGAAGATATTAGTAAGAGTCAACTGTA
GCTCAAGGGTTTGAGCTACGAACTGCATGCCATCATCACCTCCT

SEQ ID NO. 22 translated
MNKTLTFLTVVYAVVLAQGVMDLFGEEGREEHRRHHRHSLLPPYLHNVSCVAKWEYF
KILGNRSLTFAEKKEKISEWAKKYNVVDEVASYNACREKLKQEHRKNVSEIVSNLPN

AVKKVNDLLDNENQTPRQLYVALRKLGKQNPALYRVVEYINVLVRLRREEFDEDQRR
SLSALPFGDNNDDLEEQDFGEQDFRYIYGFECARFILQNGRMFGINTDRRY

Each of the proteins provided by SEQ ID NO: 1, 3, 5, 7 and 9A-22A (or encoded by the nucleic acid sequences of SEQ ID NOS: 2, 4, 6, 8 and 9-22) may be classified as a member of the "keratin-like" proteins although, because they lack the glycine-rich domains characteristic of other KLP proteins and are localised to the gut of larval cyathostomin, the inventors have chosen to designate these proteins **cy**athostomin **g**ut-**a**ssociated **l**arval **a**ntigens (Cy-GALA).

Using any of the Cy-GALA sequences described herein, one of skill in the art could readily identify related or homologous sequences in other species, such as, for example, other cyathostomin spp. etc. For example, the nucleic acid sequence encoding these proteins could be used to probe for homologous sequences in other cyathostomin species.

Other potentially useful cyathostomin larval antigens include those encoded by the following sequences, designated SEQ ID NOS: 23-44. (SEQ ID NOS: 23, 29, and 31 are amino acid sequences and SEQ ID NOS: 24-28, 30 and 32-44 are nucleic acid sequences, SEQ ID NOS: 24, 30 and 32 encoding SEQ ID NOS: 23, 29 and 31 respectively). It should be understood that the invention further encompasses proteins, peptides and amino acids having sequences encoded by SEQ ID NOS: 25-28 and 32-44.

SEQ ID NO: 23 (CID-1):

REKARIIQDEYTKRMQQVTPQAQEFLAKWEKTWFTNVQQYSGDKKAFFKQMIELIPQ
LMEEVHGFSEETWKSLEEQFPEQTAAWKDNEDRLKQFYEFIKSLPKQDLAEDPEAFR
KFAHLGLQKLLPIEALRA

SEQ ID NO: 24 (nucleic acid sequence encoding CID-1)

AGGGAGAAGGCTAGAATTATTCAAGACGAATACACTAAACGTATGCAGCAGGTCACA
CCACAAGCTCAGGAATTCCTGGCAAAATGGGAGAAGACATGGTTCACGAATGTGCAG
CAATATAGCGGAGATAAGAAAGCTTTCTTCAAGCAGATGATTGAGCTAATCCCTCAA
CTAATGGAGGAGGTTCATGGGTTCTCGGAAGAGACTTGGAAGAGCCTTGAGGAGCAA
TTCCCAGAGCAGACAGCCGCATGGAAAGATAATGAGGATCGCCTAAAGCAATTTTAT
GAGTTTATCAAGAGCCTACCCAAGCAGGACTTAGCTGAGGATCCGGAAGCATTCAGA
AAGTTCGCTCACCTCGGACTCCAGAAACTTCTTCCAATTGAAGCTCTCAGAGCT

CID antigens from other Cyathostomin organisms may include those encoded by the genomic DNA sequences provided as SEQ ID NOS: 25-28 provided below.

SEQ ID NO: 25

C.cat01-CID

TGGTCACACCACAAGCTCAGGAGTTCCTGGCCAAGGTAAGCTATTACCTTACCAGGGTGAGGGGAAAGA
AGTTGGCAGCGGTCGGAAACCCGGTAATCTACTGACTTTACCAATTATTTTCAGTGGGAGAAGACATGG
TTCACGAATATACAGCAATACAGTGGAGACAAGCAAGCCTTCTTTAAGCAGATGATTGAACTAATTCCT
CAACTTATGGAGGAGGTTCAGGTAAGTTAGCCGCAAAAATTTTTAACCAATGGTTGAGCTCGACATTTT
TTCAGGGATTCACAGAGGAGACTTGGAATAGCCTGAGGGAGCAATTCCCGGAGCAGACAGCCGCATGGA
AGGATCGTGAGTATCTTTCATAATTACTGTACTTGGAATTATACTTTACAATCATAATCCTACTCTTAG
ACGAGGATCGCCTGAAGCAATTCTATGAGTTCATTAAGAGCCTACCCAAACAACAATTAGCTGAGGTGA
TTTTCATTGATTTTTCGAAAAATATATTTTTGATACATTCTTTTTCAGGATCCGGAAGCTTTCAGAAAG
TTCGCTCACCTCG

SEQ ID NO: 26

C.cat02-CID

TTGTCACACCACAAGCTCAGGAGTTCCTGGCTAAGGTAAGCTATTACCTTACCAGGGTGAGGGGGAAGA
AGTTGGGAGCGGTCGGAAACCCGGTAATCTACTGACTTTACCAATTATTTTCAGTGGGAGAGGACATGG
TTCACGAATATACAGCAATACAGTGGAGACAAGCAAGCCTTCTTTAAGCAGATGATTGAACTAATTCCT
CAACTTATGGAGGAGGTTCAGGTAAGTTGGCCGCAAAAATTTTTAACCAATGGTTGAGCTCGACATTTT
TTCAGGGATTCACAGAGGAGACTTGGAATAGCCTGAGGGAGCAATTCCCGGAGCAGACAGCCGCATGGA
AGGATCGTAAGTATCTTTCATAATTACTGTACTTGGAATTATACTTTACAATCATAATCCTACTCTTAG
ACGAGGATCGCCTGAAGCAATTCTATGAGTTCATTAAGAGCCTACCCAAACAACAATTAGCTGAGGTGA
TTTTCATTGATTTTTCGTACGAAAAATATATTTTTGATACATTCTTTTTCAGGATCCGGAAGCTTTCAG
AAAGTTCGCTCACCTCG

SEQ ID NO: 27

C.lon91-CID

AGGTCACACCACAAGCTCAGGAATTCCTGGCAAAGGTAAGCTATCACCTTACCAGGGTGAGGGGTAGAA
GTTAGGAGCGAGGGAACCCGGTGATCTCTTATACCCATTACTTCAGTGGGAGAAGATATGGTTCACGAA
TGTACAGCAATATAGTGGAGACAAGCAAGCCTTCTTCAAGCAGATGATTGAACTAATTCCTCAACTTAT
GGAGGAGGTACAGGTAAGTCAGCTAAAGTGATTTTAAGAAAAAATTAAGCCTGATTTTCCTTTCAGGGA
TTCTCAGAGGAGACTTGGAATAGCCTTAAGGAGCAATTCCCTGAGCAGACAGCCGCATGGAAGGATAGT
GAGTATTTTTCATAATTACTGTACTTGGAATTATACTTTACAATCATAATCCTACCCTCAGACGAGGAG
CGCCTGAAGCAATTCTATGAGTTCATTAAGAGCCTACCCAAACAACAAATAGCTGAGGTGATTTTCATT
GATTTTTCGTACGAAAAGTATATTTTTAATACATTCTTTTGCAGGATCCGGAAGCCTTCAGAAAGTTCG
CTCACCTCG

SEQ ID NO: 28

C.nas07-CID

AGGTCACACCACAAGCTCAGGAATTCCTGGCAAAGGTAAGCTACCATATTTCGAGGGGGAGGGCAATTT
TGGAGCGAGGGAGGAGAGGAAAGGGAGAGAAACACTGGTTGGGATCACTAACTCTACCCGCCACTTCCA
GTGGGAGAAGACATGGTTCACGAATGTGCAGCAATATAGCGGAGATAAGAAAGCCTTTTTCAAACAGAT
GATTGAGCTAATCCCTCAACTAATGGAAGAGGTTCATGTAAGTCAACCAAAGTGGCTTTTAAGCGGAGA
TTAAACTCGAATTTTTCTTCAGGGGTTCTCGGAGGAGACTTGGAAGAGCCTTGAGGAGCAATTCCCAGA
GCAGACAGCCGCATGGAAGGATAGTAAGCATTCTTCATAGCTCCCGCCTTTATCATTTATCTTCACGAT
AGTAATCTTATTTTTAGATGAGGATCGCCTGAAGCAATTTTATGAGTTCATCAAGAGCCTACCCAAGCA
GGACTTAGCTGAGGTAACTTTCATGGTTTTTTCCTGAGCTGTAAAAATGCTTGCAACTAACAACTTTTC
TAGGATCCGGAAGCTTTCAGAAAGTTCGCTCACCTCG

SEQ ID NO: 29 (FAR-2):

KKESQGFFSIPVDNLRASPFLLQYIKEYIPDYKNAMEKFEDIPKQYRDLIPEEV
ATHLKAITAEEKAVLKEVMKDYAKYKDEEEFLKALKEKSEGLHEKASKLHN
FIKGKVDALGDEAKAFVKKVIAAAREVHAKLLAGDKPSLEDIKKKAKEHMG
EFEKLSDDAKEDLKKNFPILTSVWTNEKTRALIDKYVEN

SEQ ID NO: 30 (nucleic acid sequence encoding (FAR-2)

ATGCTTCGAATAACTTTCTTCCTTGCTCTCTTTGTTGTCTACACTTTTTCTGCACCC
TCTGGACCCGCTGAAGAGAAGATAGATGTGGAAAAAATGGAAAAATTTGAAGATATT
CCAAAGCAATATCGAGACCTTATTCCGGAAGAGGTAGCTACACACCTCAAAGCCATC
ACCGCTGAAGAGAAAGCTGTTCTAAAAGAGGTAATGAAGAATTATGCAAAGTACAAG
AACGAGGAGGAGTTTTTGGAAGCGTTGAAAGAAAAATCAGAGAGTTTGCATGAGAAA
GCCAGCAAACTTCACAATTTTATCAAAGGGAAGGTTGACGCACTTGGAGATGAAGCA
AAGGCATTTGTGAAGAAGGTTATCGCAGCTGCTCGAGAAGTGCATGCCAAACTTCTT
GCCGGGGACAAACCATCGCTTGAAGATATCAAGAAGAAAGCCAAGGAGCATATGGCT
GAATTCGAGAAACTAAGCGATGATGCCAAGGAGGATCTCAAAAAGAATTTCCCAATC
CTTACTTCCGTCTGGACAAATGAGAAAACAAGAGCGTTGATTGACAAATATGTGGAG
AAC

SEQ ID NO: 31 (UNK-50a):

GKMSDLWTAISETNKVRLFNTLSLGIAGVLCITTAFIPVENQVVCAVLITLLQG
VIGFNSAGYNKAAVIVARQHAHLLLTCFGLIVTFVPLVQPFIVQLVAPDHSWD
QWFYLFVGHGLVLVIANLFFCLTIEAKPAAFTQKTDSS

SEQ ID NO: 32 (nucleic acid sequence encoding UNK-50a)

GGTAAAATGTCAGATTTATGGACGGCAATAAGCGAAACAAATAAAGTCCGCTTGTTC
AACACCTTGTCGCTGGGAATTGCTGGCGTACTGTGTATAACTACTGCTTTCATTCCT
GTGGAAAATCAGGTTGTTTGCGCTGTTTTAATCACGTTATTGCAAGGAGTTATCGGA
TTCAATTCAGCTGGATATAACAAAGCTGCAGTCATTGTTGCTAGGCAGCATGCTCAT
CTTCTGTTGACCTGCTTTGGGCTCATTGTCACTTTTGTCCCCTTGGTGCAGCCATTC
ATAGTTCAACTTGTGGCCCCTGACCATAGCTGGGACCAATGGTTTTATCTGTTTGTT
GGGCATGGTCTCGTACTTGTTATAGCGAATTTATTCTTTTGTCTCACTATCGAGGCG
AAACCGGCAGCGTTCACACAGAAAACTGATTCATCA

The following sequences represent nucleic acid sequences encoding potentially useful EL3 antigens (or fragments or portions thereof). As above, it should be understood that the in addition to these nucleic acid sequences, the present invention relates to amino acid sequences comprising sequences encoded by SEQ ID NOS: 33-44 or derivatives, variants or homologues thereof.

SEQ ID NO: 33

EL3sequence1

GGTTTAATTACCCAAGTTTGAGGTACTTTCTAAATCTGACCCGATCAACTGATTGTGGTCTGATTAAAT
TTTGAAAATCTCTCCCTGAATAGGGAGAGTACAAGAGTGCATATCCAAAAAAAAAAAAAAAAAAAAAA
AAAAAAACATGTCGGCCGCCTCGGCCTCTAGAATA

SEQ ID NO: 34

EL3sequence2

GGTTTAATTACCCAAGTTTGAGTGTCATGAAGCTTGCCTGAAAAAAGCAGAGAAACCAAGAGGAGATAG
TTTCACAGTTCCGCCAGACAGGAAATGCGTGCCAAGATGTTTTGCGGAAGAGGAGAAACGTCGTTCACT
TAGAATGAGAAGGCATTGATTCTGTTTAGTCGTTGAGATATTTAAAAATTCTTTGCAGAAAACCTTTTC
AAATCATAAAGTCGAAGACCACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACATGTCGGCCGCCTCGG
CCTCTAGAATA

SEQ ID NO: 35

EL3sequence3 (Cy-Ins-1)

GGTTTAATTACCCAAGTTTGAGGCTGCTTCAACAGTAGGTTTAGAAATGACATCGCGGATATGGCGCCG
CACCCAGAGCCCTCCATTATTGCTACTCCTGTTGTTGATCAGTCTACCAGTAGCTGAGTGTAGTATTCG
ACTATGTGGAGTGCGACTAACACGAACTCTTATGGCTATCTGCAGGAATCAATTATGCGGTTATTCGCA
AAGTAAAAGATCTGCTATGTGGGAAGAGCCTCGACTGGAAACCGTGCACTCAACAATGAAACGATCAGG

GATCGCCACCGAATGCTGCGAGAATCGGTGCTCATTTAGCTACTTAAAGACATACTGCTGCAGCACTTA
GCCTTGGCATCTTAAGCCGCTTTTATCTCCTCTCCATGATCTCTCTTCGTTATCTGTATAACCGAATAT
AGTCATTCCGGAAATGCGGATGCTTAGGCCAATTTGTTGACGTTTGCCGCATGAATCATTTGCTGTTCG
TCATTATCTCACAGACGTGTAAAAGATCTCTTTTTATGAAAGTCTATTTTGTTTGAGCTGCACCATTAA
ACCGTTCACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACATGTCGGCCGCCTCGGCCTCTAGAATAA

SEQ ID NO: 36

EL3sequence4

GGTTTAATTACCCAAGTTTGAGGTACTTTCTAGATCTGACCCGATCAACTGATTGTGGTCTGATTAAAT
TTTGGAAATCTCTTCCTGAACAGGGAGAGTACAAGAGTGTATATGAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAACATGTCGGCCGCCTCGGCCTCTAGAATA

SEQ ID NO: 37

EL3sequence5

GGTTTAATTACCCAAGTTTGAGGATGCTTAGTTTCAAGCTCGTTCTTCTCTTCGTACTTCTCACAGCTT
GTGTGCTAACAGATCCAAGAGTGTTAATCCGAGAAAAGCGAATGGACTGGAGACGTTACTATAGCAGAT
GGGGTCGCGGAAGCTCTAATTGGGGAAACCGCGGAGGTACCTTCGGCGGACGAAAATGGAGTTACCCGA
CTTTTGGACAATGGGGACATTAACATCTGATGTATGAAAAGATCTAATGAAATAAAGCTTCGAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAACATGTCGGCCGCCTCGGCCTCTAGAATA

SEQ ID NO: 38

EL3sequence6 (Cy-Cbg-1)

GGTTTAATTACCCAAGTTTGAGAATGTTCGAAAAATTCCTTCTGCTACTGATCGTTGTGATCGCCCTCA
TTTCTTTGGCGTCTGCAGATTTTTCATGCTTCTTCGGTGATACCATCTGCAAGAGCATTACATGCAGGG
GCTGCACCGTCGCCACTTGCCTTAATGGAGACTGTATGTGCACACTATGTAACTGATGATCTTCACATG
TCGCATTACCATTTGTAACAAATACATTTTCTCTTGTTCATAATAAATTTTTCACTCAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAACATGTCGGCCGCCTCGGCCTCTAGAATA

SEQ ID NO: 39

EL3sequence7

GGCCGCGGGATTTTCTAGAGGCCGAGGCGGGTTTTAGGTTGTTCCTCAAACTTGGGTAATTAAACCACG
AGGCCGAGGCGGGTTTTAGGTTGTTCTCAAACTTGGGTAATTAAACCACGATGGCGAGGCGGGTTTTAG
GTTGTTCTCAAACTTGGGTAATTAAACCACGATGGCGAGGCGGGTTTTAGGTTGTTCCTCAAACTTGGG
TAATTAAACCAAGAGGCCGAGGCGGGTTTTAGGTTGTTCCTCAAACTTGGGTAATTAAACCACGATGGC
GAGGCGGGTTTTAGGTTGTTCTCAAACTTGGGTAATTAAACCAATCACTAGT

SEQ ID NO: 40

EL3sequence8

GGCCGCGGGATTATTCTAGAGGCCGAGGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGGGGAGAG
GGAAAAGTTTCTTTTCTCTCGGATACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAACATGTCGGCCGC
CTCGGCCTCTAGAATA

SEQ ID NO: 41

EL3sequence9

GGCCGCGGGATTTTCTAGAGGCCGAGGCGTCTTACTTGGGTGGCTCAATAACTGAAAGCTTAGAATTCA
TTAAACCTTAACCCACAGGGGTTATTTGACATGCTTGACTTGAAAATGATGCTCTTCTGCTTGTAGTTG
TTTTATTATGCTAGCTGTAAGTATACTCTGGTAGACCAGAACATCAATGTGCTAGTTGAATGTATCATG
TTATCACTTTGTCACACTCTATACGAATCTAGGTGTGGCAGGCCACACCCCTCTCCTGACCCTGTTCAC
CATCAATTAGCTTTTAGCTGTTATTTAATAACATCACACTGATTGCAAAAAAAAAAAAAAAAAAAAAAA
AAAAAACATGTCGGCCGCCTCGGCCTCTAAAAAATCACTAGT

SEQ ID NO: 42

EL3sequence10

GGCCGCGGGATTATTCTAGAGGCCGAGGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGAAGCAGT
GGTATCAACGCAGAGTGGCCATTACGGCCGGGTGGTGACCACGGGTGACGGGGAATTAGGGTTCGATTC
CGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GACCCGGGGAGGTAGTGACGAAAAAAAAAAAAAAAAAAAAAAAAAAAACATGTCGGCCGCCTCGGCCTC
TAGAATAATCACTAGT

SEQ ID NO: 43

EL3sequence11

GGCCGCGGGATTTTCTAGAGGCCGAGGCGGGTTTTAGCTCAAACTTGGGTAATTAAACCGGTAGGATGG
CGAGGCGGGTTTCTCAAACTTGGGTAATTAAACCAGTAGGATGGCGAGGCGGGTTTCTCAAACTTGGGT
AATTAAACCGGTAGGAGGCCGAGGCGGGTCTCAAACTTGGGTAATTAAACCAATCACTAGT

SEQ ID NO: 44

EL3sequence12

CAAGTTTGAGGTACTTTCTAGATCTGACCCGATCAACTGATTGTGGTCTGATTAAATTTTGGAAATCTC
TTCCTGAACAGGGAGAGTACAAGAGTGTATATTAAGAAAAAAAAAAAAAAAAAAAAAAAAAACATGTCGG
CCGCCTCGGCCTCTAGAATAATCACTAGT

As such, the present invention relates to the proteins encoded by the sequences designated as SEQ ID NOS: 1, 3, 5, 7, 23, 29 and 31, the corresponding gene sequences (such as, for example, those given as SEQ ID NOS: 2, 4, 6, 8, 24, 30 and 32) and proteins, peptides and/or amino acids comprising sequences encoded by SEQ ID NOS 9-22, 25-28 and 33-44, as well as any fragments, portions, mutants, variants, derivatives, analogues and/or homologoues/orthologues thereof. Furthermore, the

methods described herein may provide means for detecting levels of antibodies which bind to proteins comprising (or encode by) any of SEQ ID NOS: 1-44 (or fragments, portions, mutants, derivatives, analogues or variants thereof).

Typically the fragments, portions, mutants, variants, derivatives, analogues and/or homologoues/orthologues mentioned in this invention are immunogenic or encode immunogenic cyathostomin larval antigens – that is, they are capable of generating immune, preferably humoral, responses.

The term "mutants" may encompass naturally occuring mutants or those artificially created by the introduction of one or more amino acid/nucleic acid additions, deletions, substitutions or inversions.

One of skill in this field will readily understand that proteins or nucleic acids homologous to the proteins encoded by SEQ ID NOS: 1, 3, 5, 7, 23, 29 and 31 or nucleic acid sequences of SEQ ID NOS: 2, 4, 6, 8, 24, 25-28, 30, 32 and 33-44, may exhibit as little as 20 or 30% sequence homology or identity thereto (or to a portion thereof). In other instances however, homologous proteins or nucleic acid sequences may exhibit at least 40, 50, 60, 65 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% homologly or identity the whole or part of SEQ ID NOS: 1-14 detailed above. As such, proteins or nucleic acids homolous to (or partially identical with) the proteins and/or nucleic acid sequences provided by SEQ ID NO: 1-14 are also included within the scope of this invention.

It should also be understood that natural variations due to, for example, polymorphisms, may exist between related (or homologous) proteins/genes from any given cyathostomin species. These variants may manifest as proteins/genes which exhibit one or more amino/nucleic acid substitutions, additions, deletions and/or inversions relative to a reference sequence (for example any of the sequences provided by SEQ ID NOS: 1-44 described above). All such variants, especially those which are functional and/or are immunogenic (or encode functional/immunogenic proteins or peptides) are to be included within the scope of this invention.

Additionally, or alternatively, analogues of the various peptides described herein may be made by introducing one or more amino acid substitutions into the primary sequence. In certain embodiments, one or more of these substitutions may represent a "conservative substitution". One of skill in this field will undertsand that the term "conservative substitution" is intended to embrace the act of replacing one or more amino acids of a protein or peptide sequence with an alternate amino acid with

similar properties and which does not substantially alter the physio-chemical properties and/or structure or function of the native (or wild type) protein.

As is well known in the art, the degeneracy of the genetic code permits substitution of one or more bases in a codon without changing the primary amino acid sequence. Consequently, although the nucleic acid sequences described in this application are known to encode potentially useful cyathostomin larval antigens, the degeneracy of the code may be exploited to yield variant nucleic acid sequences which encode the same primary amino acid sequences.

Also encompassed by this invention are splice variants of the primary gene transcripts encoded by any of the gene sequences described herein, as well as and the translated Cyathostomin larval antigen splice variant proteins which are encoded thereby. By way of example, splice variants of the Cy-GALA proteins described herein, including, for example, variants encoded by transcripts having 115bp segment deletions, are within the scope of this invention. Furthermore, one of skill in this field will readily appreciate that polyadenylation variants and start codon variants, including cDNA sequences encoding the same, may also be included within the scope of this invention.

As stated, this invention finds particular application in the identification or diagnosis of cyathostomin infections in horses but may be more generally be used to diagnose or identify cyathostomin infections present in other species of the Equidae family including, for example, donkeys and zebra

The term "sample" should be understood as including any samples comprising antibodies and/or cyathostomin larval antigens. For example, suitable samples may include fluids such as whole blood, plasma, serum, saliva, sweat and/or semen. In other instances "samples" such as tissue biopsies and/or scrapings may be used. In particular biopsies or scrapings from the gut may be used. In addition, a sample may comprise a tissue or gland secretion and washing protocols may be used to obtain samples of fluid secreted into, for example, the gut. In other embodiments, faecal samples may be used. One of skill will undrerstand that in order to prepare a faecal sample for use, it may be necessary to add buffers and various protease inhibitors and subject the sample to procedures such as centrifugation, to remove particulate material. As such, "faecal samples" may represent suitable samples for use in the methods provided by this invention. As stated, a "reference" or "control" sample may

be derived from healthy animals or from animals not having high mucosal burdens of cyathostomin parasites or larval cyathostominosis.

In order to identify a level of anti-cyathostomin larval antigen antibodies present in a sample, the sample may be contacted with one or more cyathostomin larval antigen(s) (such as those provided, comprising or encoded by SEQ ID NOS: 1-44) under conditions which permit binding between any anti-cyathostomin larval antigen antibodies present in the sample and the cyathostomin larval antigen(s). Anti-cyathostomin larval antigen antibodies bound to cyathostomin larval antigen may easily be detected with the use of agents capable of binding anti-cyathostomin larval antigen antibodies. In one embodiment, the agents capable of binding anti-cyathostomin larval antigen antibodies may be conjugated or linked to a detectable moiety.

One of skill will appreciate that while the methods provided by this invention may provide a means of detecting antibodies having affinity for, or specificity/selectivity to a single cyathostomin antigen (such as any described herein), in certain embodiments, the methods may exploit the use of one or more of the cyathostomin antigens. Since, for example, horses tend to be infected with one or more different cCyathostomin species, assays/methods which utilise cocktails of cyathostomin antigens provide a means of increasing the likelihood of a positive diagnosis. Accordingly, it should be understood that the methods described herein may use one or more of the cyathostomin antigens described herein.

In one embodiment, the methods provided by this invention may utilise substrates to which one or more cyathostomin larval antigens have been bound, conjugated or immobilised. One of skill in that art will appreciate that in addition to techniques which allow antigens to be bound, conjugated or immobilised "directly" on to the surface of substrates, other techniques may involve the use of substrates which have been coated with agents capable of binding cyathostomin larval antigens.

It is to be understood that the term "agents capable of binding cyathostomin larval antigens" may include, for example, antibodies such as monoclonal or polyclonal antibodies and/or other types of peptide or small molecule capable of binding to cyathostomin larval antigens. It should be noted that this definition applies to all types of binding agent mentioned herein. Furthermore, references to "antibodies" herein are indented to encompass "anti-cyathostomin larval antigen antibodies".

The techniques used to generate antibodies (either monoclonal or polyclonal) are well known to one of skill and may involve the use of cyathostomin antigens (or fragments or portions thereof) either isolated or purified from cyathostomin parasites or recombinantly generated as described herein.

Suitable substrates may include, for example, glass, nitrocellulose, paper, agarose and/or plastics. A substrate such as, for example, a plastic material, may take the form of a microtitre plate.

In order to detect a level of antibody present in a sample, immunological detection techniques such as, for example, enzyme-linked immunosorbent assays (ELISA) may be used. One of skill in this field will appreciate that ELISAs may use substrates to which cyathostomin larval antigens have been "captured" or bound by binding agents (capable of binding cyathostomin larval antigens) bound or immobilised to the substrate. Alternatively, substrates may comprise cyathostomin larval antigens, which have been directly bound or immobilised to the substrate.

An ELISA may involve contacting the sample to be tested with a substrate under conditions which permit binding between any antibodies present in the sample and the cyathostomin larval antigens bound or immobilised to the substrate as described above. One familiar with these techniques will appreciate that prior to contacting the sample to be tested with the substrate, a blocking step may be introduced to reduce incidences of non-specific binding.

An ELISA may comprise the further step of contacting the substrate with a further binding agent capable of binding one or more of the antibodies present in the sample. Such agents may otherwise be known as "secondary antibodies" and may take the form of rodent or ruminant antibodies specific to particular forms of equine antibody.

Secondary antibodies useful in the present invention may be conjugated to moieties which permit them to be detected (referred to hereinafter as "detectable moieties"). For example, the secondary antibodies may be conjugated to an enzyme capable of reporting a level via a colourmetric chemiluminescent reaction. Such conjugated enzymes may include but are not limited to Horse Radish Peroxidase (HRP) and Alkaline Phosphatase (AlkP). Additionally, or alternatively, the secondary antibodies may be conjugated to a fluorescent molecule such as, for example a fluorophore, such as FITC, rhodamine or Texas Red. Other types of molecule which may be conjugated to binding agents include radiolabelled moieties.

The amount of secondary antibody (identifiable by means of the detectable moiety) bound to the anti-cyathostomin larval antibodies, may be representative of the anti-cyathostomin larval antibodies present in the sample tested.

Alternatively, in order to identify a level of cyathostomin larval antigen present in a sample, a substrate or substrate comprising one or more agents capable of binding one or more cyathostomin larval antigens, may first be contacted with a sample to be tested. Any cyathostomin larval antigen bound to the substrate or to the agents capable of binding the cyathostomin larval antigen, may be detected with the use of a further agent capable of binding the cyathostomin larval antigen (referred to hereinafter as the "primary binding agent"). Additionally, or alternatively, the primary binding agents may have affinity for, or bind to cyathostomin larval antigen::substrate complexes or complexes comprising cyathostomin larval antigen and the abovementioned agents capable of binding the cyathostomin larval antigen. In one embodiment, the primary binding agent may be an antibody conjugated to a detectable moiety as described above.

Alternatively, any cyathostomin larval antigen bound to the substrate or agents capable of binding the cyathostomin larval antigen, may be detected by means of a yet further binding agent having affinity for the primary binding agents. In certain embodiments, the further binding agents may be conjugated to detectable moieties.

In one embodiment, the methods for identifying a level of cyathostomin larval antigen or a level of anti-cyathostomin larval antigen antibodies, may take the form of "dip-stick" test, wherein a substrate (or portion thereof) is contacted with a sample to be tested under conditions which permit the binding of any cyathostomin larval antigen or anti cyathostomin larval antigen antibodies present in the sample, to the substrate or a binding agent bound or immobilised thereto.

Other techniques which exploit the use of agents capable of binding the cyathostomin larval antigen or antibodies which bind thereto include, for example, techniques such as western blot or dot blot. A western blot may involve subjecting a sample to electrophoresis so as to separate or resolve the components, for example the proteinaceous components, of the sample. In other embodiments, electrophoresis techniques may be used to separate proteins purified from cyathostomin parasites and/or proteins generated in a recombinant form. The resolved components/proteins may then be transferred to a substrate, such as nitrocellulose.

In order to identify any cyathostomin larval antigen present in a sample, the substrate (for example nitrocellulose substrate) to which the resolved components and/or proteins have been transferred, may be contacted with a binding agent capable of binding cyathostomin larval antigens under conditions which permit binding between any cyathostomin larval antigen in the sample (or transferred to the substrate) and the agents capable of binding the cyathostomin larval antigen.

Advantageously, the agents capable of binding the cyathostomin larval antigen may be conjugated to a detectable moiety.

Additionally, the substrate may be contacted with a further binding agent having affinity for the binding agent(s) capable of binding the cyathostomin larval antigen. Advantageously, the further binding agent may be conjugated to a detectable moiety.

Similar techniques may also be used to detect levels of anti-cyathostomin larval antigen antibodies present in samples. Techniques of this type may be known as "immunoblots" or "dotblots" or 'dipsticks' where cyathostomin antigen(s) is/are immobilised onto suitable substrates (for example a nitrocellulose substrate) and contacted with agents capable of binding cyathostomin antigen(s). In certain embodiments any of the samples described above may be used a source of cyathostomin antigen. Additionally or alternatively, the cyathostomin larval antigen may be isolated or purified from the parasite, or produced in recombinant form.

Other immunological techniques which may be used to identify a level of cyathostomin larval antigen in a sample include, for example, immunohistochemistry wherein binding agents, such as antibodies capable of binding cyathostomin larval antigens, are contacted with a sample such as those described above, under conditions which permit binding between any cyathostomin larval antigen present in the sample and the cyathostomin larval antigen binding agent. Typically, prior to contacting the sample with the binding agent, the sample is treated with, for example a detergent such as Triton X100. Such a technique may be referred to as "direct" immunohistochemical staining.

Alternatively, the sample to be tested may be subjected to an indirect immunohistochemical staining protocol wherein, after the sample has been contacted with a cyathostomin larval antigen binding agent, a further binding agent (a secondary binding agent) which is specific for, has affinity for, or is capable of binding the

cyathostomin larval antigen binding agent, is used to detect cyathostomin larval antigen /binding agent complexes.

The skilled person will understand that in both direct and indirect immunohistochemical techniques, the binding agent or secondary binding agent may be conjugated to a detectable moiety. Preferably, the binding agent or secondary binding agent is conjugated to a moiety capable of reporting a level of bound binding agent or secondary binding agent, via a colourmetric chemiluminescent reaction.

In order to identify the levels of cyathostomin larval antigen present in the sample, one may compare the results of an immunohistochemical stain with the results of an immunohistochemical stain conducted on a reference sample. By way of example, a sample revealing more bound cyathostomin larval antigen binding agent (or secondary binding agent) than in a reference sample, may have been provided by a subject with a cyathostomin infection.

In addition to the methods and techniques described above, the present invention also contemplates the use of a range of PCR based techniques which may be used to detect levels of cyathostomin antigen gene expression or gene quantity in a given sample. Useful techniques may include, for example, polymerase chain reaction (PCR) using genomic DNA as template or reverse transcriptase (RT)-PCR (see below) based techniques in combination with real-time PCR (otherwise known as quantitative PCR). In the present case, real time-PCR may used to determine the level of expression of the genes encoding any of the cyathostomin larval antigens described herein. Typically, and in order to quantify the level of expression of a particular nucleic acid sequence, RT-PCR may be used to reverse transcribe the relevant mRNA to complementary DNA (cDNA). Preferably, the reverse transcriptase protocol may use primers designed to specifically amplify an mRNA sequence of interest (in this case a cyathostomin mRNA encoding a cyathostomin larval antigen). Thereafter, PCR may be used to amplify the cDNA generated by reverse transcription. Typically, the cDNA is amplified using primers designed to specifically hybridise with a certain sequence and the nucleotides used for PCR may be labelled with fluorescent or radiolabelled compounds.

One of skill in the art will be familiar with the technique of using labelled nucleotides to allow quantification of the amount of DNA produced during a PCR. Briefly, and by way of example, the amount of labelled amplified nucleic acid may be

determined by monitoring the amount of incorporated labelled nucleotide during the cycling of the PCR.

Further information regarding the PCR based techniques described herein may be found in, for example, PCR Primer: A Laboratory Manual, Second Edition Edited by Carl W. Dieffenbach & Gabriela S. Dveksler: Cold Spring Harbour Laboratory Press and Molecular Cloning: A Laboratory Manual by Joseph Sambrook & David Russell: Cold Spring Harbour Laboratory Press.

Other techniques that may be used to determine the level of cyathostomin larval antigen gene expression in a sample, include, for example, northern and/or Southern blot techniques. A northern blot may be used to determine the amount of a particular mRNA present in a sample and as such, could be used to determine the amount of cyathostomin larval antigen gene expression. Briefly, total or messesnger (m)RNA may be extracted from any of the samples described above using techniques known to the skilled artisan. The extracted RNA may then be subjected to electrophoresis. A nucleic acid probe, designed to hybridise (i.e. complementary to) an RNA sequence of interest – in this case the mRNA encoding a cyathostomin larval antigen, may then be used to detect and quantify the amount of a particular mRNA present in a sample.

Additionally, or alternatively, a level of cyathostomin larval antigen gene expression may be identified by way of microarray analysis. Such a method would involve the use of a DNA micro-array which comprises nucleic acid derived from cyathostomin larval antigen genes. To identify a level of cyathostomin larval antigen gene expression, one of skill in the art may extract the nucleic acid, preferably the mRNA, from a sample and subject it to an amplification protocol such as, RT- PCR to generate cDNA. Preferably, primers specific for a certain mRNA sequence – in this case sequences encoding cyathostomin larval antigen genes may be used.

The amplified cyathostomin larval antigen cDNA may be subjected to a further amplification step, optionally in the presence of labelled nucleotides (as described above). Thereafter, the optionally labelled amplified cDNA may be contacted with the microarray under conditions which permit binding with the DNA of the microarray. In this way, it may be possible to identify a level of cyathostomin larval antigen gene expression.

In addition, other techniques such as deep sequencing and/or pyrosequencing may be used to detect cyathostomin larval antigen sequences in any of the samples

described above, particularly faecal matter extracts. Further information on these techniques may be found in "Applications of next-generation sequencing technologies in functional genomics", Olena Morozovaa and Marco A. Marra, Genomics Volume 92, Issue 5, November 2008, Pages 255-264 and "Pyrosequencing sheds light on DNA sequencing", Ronaghi, Genome Research, Vol. 11, 2001, pages 3-11.

The present invention also extends to kits comprising reagents and compositions suitable for diagnosing cyathostomin infections. For example, depending on whether or not the kits are intended to be used to identify levels of cyathostomin larval antigen or antibodies thereto in samples, the kits may comprise substrates having cyathostomin larval antigens or agents capable of binding cyathostomin larval antigens, bound thereto. In addition, the kits may comprise agents capable of binding cyathostomin larval antigens – particularly where the kit is to be used to identify levels of cyathostomin larval antigens in samples. In other embodiments, the kit may comprise agents capable of binding the cyathostomin larval antigens, for example specifically raised polyclonal antibodies or monoclonal antibodies. Where the kits are intended to diagnose equine cyathostomin larval infections, these binding agents may take the form of antibodies capable of binding equine antibodies. The antibodies may be conjugated to detectable moieties. Kits for use in detecting the expression of genes encoding cyathostomin larval antigen gene may comprise one or more oligonucleotides/primers for detecting/amplifying/probing cyathostomin larval antigen encoding sequences. The kits may also comprise other reagents to facilitate, for example, sequencing, PCR and/or RFLP analysis. All kits described herein may further comprise instructions for use.

It will be appreciated that the uses, medicaments and methods of treatment described herein may require the generation of recombinant cyathostomin larval antigens (or genes encoding the same) and as such, the present invention further contemplates methods of generating and/or expressing recombinant cyathostomin larval antigen genes and/or proteins (such as for example those described above as SEQ ID NOS: 1-44). One of skill in this field will appreciate that PCR techniques may be exploited to selectively obtain cyathostomin larval antigen gene sequences from a variety of sources including, for example, equine gut tissue, faecal matter or extracts prepared from cyathostomin nematodes. In one embodiment, molecular cloned cyathostomin larval antigen gene sequences may be introduced into a vector (such as a plasmid or expression cassette). In one embodiment, the vector may further

comprise a nucleotide sequence of a tag or label to assist in protein purification procedures.

A host cell may be transformed with the vector and maintained under conditions suitable to induce expression of the cyathostomin larval antigen gene sequence and production of recombinant cyathostomin larval antigen. Techniques used to purify recombinant proteins generated in this way are known and, where the recombinant protein is tagged or labelled, these may include the use of, for example, affinity chromatography techniques.

In view of the above, further aspects of this invention provide an expression vector comprising a cyathostomin larval antigen gene sequence and a host cell transformed therewith, respectively.

In a further aspect, the present invention provides a method for determining whether or not an equine subject should be treated with anthelmintic drug, said method comprising the step of detecting a level of anti- cyathostomin larval antigen antibodies in a sample as per the first aspect of this invention and/or a level of cyathostomin larval antigen in a sample, wherein a level of anti-cyathostomin larval antigen antibodies and/or antigen, is indicative of an equine subject that should be administered a anthelminitc drug. In one embodiment, the anthelminitc drug may be Moxidectin.

DETAILED DESCRIPTION

The present invention will now be described in detail and with reference to the following Figures which show:

Figure 1. ClustalW alignment of Cy-GALA-1 with its orthologues in other nematode species. Cyathostomin (Cy) GALA-1 is compared to *N. brasiliensis* keratin-like protein (Nb-KLP) (accession number: BAB68205); *T. circumcincta* (Tc) (AAM45145); *O. ostertagi* (Oo) (CAD22110); *C. elegans* (Ce) KLP-1 (NP_502026) and Ce-KLP-2 (NP_501448). The signal peptide for each sequence is underlined and the domain of unknown function (DUF148) is boxed. The histidine-rich region is highlighted in grey and the glycine-rich regions of the *C. elegans* sequences are shown in bold.

Figure 2: Development transcription pattern of *Cy-gala-1*. RT-PCR was performed using gene-specific primers for *Cy-gala-1* and the housekeeping gene cytochrome oxidase c subunit I (*coxI*), from mixed-species pools of EL3 (lane 1), DL (lane 2) and

LP (lane 3) cDNA. For each reaction no-template controls were performed (N). Sizes in base pairs (bp) are labelled on the left-hand side.

Figure 3. Immunoreactivity of rCy-GALA-1. IgG(T) reactivity to rCy-GALA-1 in horses infected with cyathostomins or other helminths as assessed by (A) immunoblot and (B) ELISA. Fig. 3A. Lane 1: Coomassie blue. Lanes 2-11: IgG(T) reactivity of specific equine sera: HF (2); CI (3); a pool of sera from cyathostomin-free horses (n= 5) from an abattoir (4); a pool of sera from cyathostomin-infected horses which harboured total mucosal larval burdens of >100,000 (n = 6) from an abattoir (5); horses mono-specifically infected with *P. equorum* (6), *S. edentatus* (7), *S. westeri* (8) or *S. vulgaris* (9). Also shown is IgG reactivity in sera from a rabbit before (lane 10) and after two immunisations (lane 11) with a 20 kDa complex purified from EL3/DL somatic extracts [11]. Fig. 3B. ELISA indicating IgG(T) reactivity to rCy-GALA-1 antigen in equine sera over an experimental infection [29]. Responses in the CI group are depicted by the solid lines and black shapes and in the HF group by dashed lines and white shapes.

Figure. 4: Reactivity of anti-rCy-GALA-1 antiserum to cyathostomins and other equine helminths. IgG(T) responses were assessed by (A) ELISA and (B) immunoblot. ELISA results depict binding of anti-rCy-KLP-1 anti-sera (black) and pre-immunisation serum (white). For both assays, the antigens were as follows: 1 = rCy-GALA-1; 2 = cyathostomin IL3; 3 = cyathostomin EL3; 4 = cyathostomin DL; 5 = cyathostomin LP; 6 = adult *A. perfoliata*; 7 = adult *P. equorum*; 8 = adult *S. edentatus*; 9 = adult *S. vulgaris*; 10 = adult *S. equinus*.

Figure 5: Immunolocalisation of Cy-GALA. Transverse sections of DL cyathostomins were probed with anti-rCy-GALA-1 antiserum (A) and pre-immunization serum (B). Specific binding of antiserum in the parasite gut is indicated by the black arrows. The vertical bar represents 40μm.

Figure 6: Schematic representation of *Cy-gala-1* and the 220 bp fragment of the gene amplified from 10 cyathostomin species. *Cy-gala-1* cDNA sequence is represented by black boxes (A). The 220 bp region PCR amplified from genomic DNA samples from 10 cyathostomin species is represented by the white box. The latter is expanded to indicate the position of the intron (hatched box). The range in interspecies variation for the whole gene fragment (and also without the intron sequence) are depicted. A representative PCR product of *Cy-gala-1* is shown for each species (B): *C. catinatum* (1); *C. nassatus* (2); *C. goldi* (3); *C. longibursatus* (4); *C. coronatum* (5); *C.*

pateratum (6); *C. ashworthi* (7); *C. leptosomum* (8); *C. minutus* (9) and *C. labiatus* (10).

Figure 7 A: Optimisation of antigen cocktails. The antibody response of encysted cyathostomin infected (positive) and non-infected (negative) animals is shown for varying concentrations of antigen and two different cocktails of antigen (CT1 and CT2). CT1 contains Gala 1, Gala 2, Gala 3. CT2 contains Gala 1, Gala 2, Gala 3 and CID 1. Individual antigen concentration is shown on the x axis and optical density (O.D) on the y axis. 7B: Ratio of signal for encysted cyathostomin infected (positive) to uninfected (negative) animals in an ELISA. Individual antigen concentration is on the x axis and ratio of positive to negative optical density on the y axis. C: shows mean serum antibody response to cocktail 1 (CT1) in groups of horses with varying infection levels. CT1 contains Gala 1, Gala 2, Gala 3. Horses were grouped as follows according to total mucosal parasite burden (TMB). Neg; uninfected horses TMB = 0 (n=5),Low; TMB = 0-20000, (n=8), Medium; TMB=20000-100000, (n=7), High; TMB = >100000 (n=26). Error bars show +/- standard error of the mean. O.D = optical density. D: shows mean serum antibody response to cocktail 2 (CT2)in groups of horses with varying infection levels. CT2 contains Gala 1, Gala 2, Gala 3 and CID 1. Horses were grouped as follows according to total mucosal parasite burden (TMB). Negative; uninfected horses TMB = 0 (n=5),Low; TMB = 0-20000, (n=8), Medium; TMB=20000-100000, (n=7), High; TMB = >100000 (n=26). Error bars show +/- standard error of the mean. O.D = optical density.

Figure 8A-E: ROC analysis of ELISA data derived from cocktail (CT) 1 (which includes GALA-1, 2 and -3) and CT2 (which includes GALA-1,-2, -3 and CID-1). The Areas Under the Curve (AUC) are shown on each graph for each CT at the specified cyathostomin burden cut-off value indicated on each set of charts. The results indicate that CT1 and CT2 allow clear discrimination at different levels of cyathostomin mucusal burden, especially developing larval (DL) burdens above 120,000; however, it is likely that the AUC values could be improved by developing the assay to take into account cyathostomin species complexity and by including proteins that specifically relate to EL3. These additional proteins have been identified and will be added systematically to the cocktails to test their effect on AUC in the ROC analysis.'

MATERIALS AND METHODS

Parasite material

Cyathostomins were collected from equine large intestinal tissue as described previously [9]. Briefly, caecum and ventral colon sanmples were removed at an abattoir and luminal parasites (LP), consisting of fifth stage larvae and adults, were collected from intestinal washings using sieves. Mucosal larval stages were recovered by pepsin-HCl digestion [9]. The mucosal parasites were separated into two populations based on size following previous recommendations [13]: (i) EL3 and (ii) late third stage (LL3)/developing fourth stage (DL4), collectively termed developing larvae (DL). Nematode samples for RNA extraction were placed into RNA*later* (Ambion) at 4°C, while those for protein extraction and genomic DNA isolation were snap frozen in liquid nitrogen and stored at -80°C. For immunolocalisation experiments, DL were fixed in 10% formal saline. Infective third-stage larvae (IL3) were collected from horse faeces as described previously [8]. Individual adult cyathostomins were identified to species according to published recommendations [16]. Adult stage large strongyles, *Anoplocephala perfoliata* and *Parascaris equorum,* were also obtained and stored at -80°C.

Construction of a complementary (c)DNA library and immunoscreening

Cyathostomin RNA was extracted from DL populations by homogenisation in a mortar and pestle under liquid nitrogen, then using TRIzol (Invitrogen) according to the manufacturer's instructions. Integrity of RNA samples was assessed using a 2100 Bioanalyser (Agilent Technologies) and RNA stored in RNase-free water at -80°C. A mixed-species DL cDNA library was constructed using a SMART cDNA Library Construction Kit (Clontech Laboratories, Inc) using long distance PCR according to manufacturer's instructions. Briefly, the cDNA was synthesised by reverse transcriptase (RT)-PCR using 1μg total RNA pooled from 11 separate DL RNA samples collected over a 6-month period from a range of intestinal sites. This was done to maximise cyathostomin species representation within the cDNA library. After ligation into the λTriplEx2 vector, the cDNA was packaged into Gigapack Gold III packaging extract (Stratagene) and amplified in *Escherichia coli* XL1-Blue strain, (Stratagene). Library quality was assessed by analysing insert size in 40 plaques chosen at random. Length and identity of the inserts were determined by PCR and sequencing; the majority of plaques contained an insert with an average size of 500 base pairs (bp).

An EL3 cDNA library was constructed using the same method as for the construction of the DL cDNA library with the exception being the use of a SL1 primer to amplify nematode specific DNA prior to ligation into the TriplEx2 vector. (Martin, et al, 1995). Briefly, the cDNA was synthesised by reverse transcriptase (RT)-PCR using 1µg total RNA pooled from EL RNA samples from EL3 larvae collected from a range of intestinal sites from 6 individual horses. This cDNA was then used in a PCR with SL1 forward primer sequence: GGTTTAATTACCCAAGTTTGAG and reverse primer sequences: ATTCTAGAGGCCGAGGC and TTCTAGAGGCCGAGGCG. Products of this PCR were then used for packaging into the TriplEx2 vector as described for generation of the DL cDNA library.

Immunoscreening was performed according to the manufacturer's protocol. For immunoscreening, two types of sera were used: cyathostomin-infected (CI) and helminth-free (HF) sera [29]. Ponies in the CI group (n=3) had been trickle infected with a total of 3.9 million cyathostomin IL3 over a period of 9 weeks, while the HF control group (n=3) were maintained helminth-free. Serum was obtained weekly from both groups. For immunoscreening, a pool of CI sera was prepared by combining samples obtained from the three ponies at 12, 13, 14 and 16 weeks PI. The pool of HF sera was made by combining samples obtained from the three ponies at 2, 3, 4 and 6 weeks before the start of the infection period. To reduce background reactivity, both pools of sera were pre-absorbed with *E. coli* lysate by incubating equal volumes of each and rocking for 4 h at room temperature [37]. After centrifugation at 18,000 x g for 10 min, the supernatant was retained for probing library filter lifts. The primary immunoscreen consisted of approximately 108,000 cDNA clones in *E. coli* XL1-Blue strain. Plaque lifts were made onto nitrocellulose filters (Hybond-C Extra, GE Healthcare). The membranes were washed [five X 10 min in Tris-buffered saline (10 mM Tris, 150 mM NaCl, pH 7.4) containing 0.05% Tween-20 (TBST)], then blocked for 1 h with 1% gelatin/TBST. In the first screen, the serum pool from the CI ponies was used at 1:200 in TBST and incubated with the membranes overnight at 4°C. The secondary antibody (goat anti-equine IgG(T), Serotec) and tertiary antibody (rabbit anti-goat[IgG]:HRP, Sigma), were incubated at 1:200 and 1:500 respectively, for 1h each, with washing (as above) between steps. Filters were developed using SIGMA*FAST* DAB with Metal Enhancer (Sigma). Positive clones were isolated by taking agar plugs from the corresponding plate. Plaques that reacted non-specifically with equine sera (false positives) were identified by performing a second screen.

Here, clones selected in the first round were screened as described above, except that filters were cut in half and one half probed with the CI serum pool and the other with the HF serum pool. Only plaques that reacted with the CI serum pool and not the HF serum pool were selected for sequence analysis. Vector-specific primers were used to amplify selected phage inserts and the PCR products purified using a QIAquick PCR Purification Kit (Qiagen). Each purified PCR product was sequenced using a commercial service (MWG Biotech). The resultant sequences were translated and searched against the GenBank 'non-redundant protein' database using BLASTp, and then against the 'non-human, non-mouse' EST database using tBLASTn, from the National Centre for Biotechnology Information [3]. Sequence alignments were performed using ClustalW2 [21] from the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/clustalw2/) and analysis for signal peptides performed using SignalP 3.0 [5]. Sequence identities were calculated using MegAlign 8.0.2 (DNASTAR) based on ClustalW alignments. Molecular mass estimations were made using an online tool from the Sequence Manipulation Suite (http://www.bioinformatics.org/sms2/protein_mw.html) and glycosylation sites identified using ExPASy Prosite (http://ca.expasy.org/prosite/).

RT-PCR to determine temporal transcription pattern of the mRNA encoding the cyathostomin gut-associated larval antigen-1 (Cy-GALA-1)

Stage-specific cDNA was synthesised from 1µg each of EL3, DL and LP total RNA using a SMART cDNA Library Construction Kit (Clontech Laboratories, Inc.). Briefly, first-strand cDNA was synthesised and amplified using the long-distance PCR method (22 cycles). Double-stranded cDNA was purified using a QIAquick PCR Purification Kit (Qiagen), eluted in 50 µl dH_2O and stored at -20°C until required. Integrity and loading of each cDNA population was assessed by amplifying a portion of the cytochrome oxidase c subunit I (coxI) gene using primers designed to conserved sequences among cyathostomins (sense: 5'–AAAAAGGAGGTGTTTGGTTC–3'; antisense: 5'–CTTGAATTTGATAAAACTACACC–3'). PCR conditions were as follows: 0.3 µM primers, 0.25 µM dNTPs and 1.5 mM $MgCl_2$ with the following cycling: 94°C for 5 min, 40 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, with a final extension at 72°C for 7 min. PCR was performed using Platinum Taq (Invitrogen) with 1µl cDNA from each developmental stage. Primers were designed for the most

abundant immunoreactive clone identified in Section 2.2 and designated cyathostomin gut-associated larval antigen-1 (Cy-GALA-1). The primer sequences were as follows: sense, 5'–AATTGTGGGGAACAGGAG–3'; antisense, 5'–AATGAAAATCAGACTCCTAGG–3'. PCR conditions were as above, but using 35 cycles. This experiment was repeated twice and the PCR products were analysed on 2% w/v agarose gels using TrackIt 100 bp DNA Ladder (Invitrogen) for size determination. The gels were stained with 1 x GelRed (Biotium).

Expression of recombinant Cy-GALA-1

The Cy-GALA-1 clone from the library immunoscreen that contained the largest insert was chosen for expression of recombinant protein. This clone incorporated the full-length coding sequence of *Cy-gala-1* including the putative initiating methionine, signal peptide and poly-A tail. Primers were designed to amplify the coding sequence of *Cy-gala-1* (minus the sequence that encoded the signal peptide) for sub-cloning into pET-22b(+) vector (Novagen). Appropriate sequences encoding flanking restriction enzyme sites were incorporated for uni-directional cloning. The primer sequences were as follows (NB: *BamH*1 and *Hind*III sites underlined): sense 5'–AATTCGGATCCGCAAGGTGTCATGGACCTTTTTG–3'; antisense, 5'–CCGCAAGCTTATATCTTTCATCTGTGTTGAGTCCAAAC–3'. The PCR step was performed as described above except that the annealing temperature was 58°C and 30 cycles were used. The PCR product was purified as described above. The pET-22b(+) vector and PCR product were digested with *BamH*1 and *Hind*III and ligation, using a 1:1 ratio of vector to PCR product, performed according to Novagen's protocol. Plasmids were transformed into *E. coli* JM109 Competent Cells (Promega) following manufacturer's instructions and selected on ampicillin-agar. A selection of colonies was subjected to colony PCR to ensure the presence of the cDNA encoding Cy-GALA-1. A colony which contained an insert of the correct estimated size was subjected to plasmid purification using a Wizard Plus SV Miniprep kit (Promega) and the purified plasmid was both sequenced and transformed into *E. coli* BL21-CodonPlus (DE3)-RIL competent cells (Stratagene) for expression of recombinant protein (rCy-GALA-1). Following induction with 1mM isopropyl-beta-D-thiogalactopyranoside (Bioline), soluble rCy-GALA-1, present in the bacterial lysate supernatant, was purified on a His-trap HP column (GE Healthcare), following manufacturer's instructions. The purified protein was dialysed into phosphate buffered saline, pH 7.4 (PBS), using cellulose dialysis tubing (Sigma) and stored at -

20°C until required. Purified rCy-GALA-1 (0.5 μg) was separated by SDS-PAGE, and a band at the expected size excised and subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF-ToF) mass spectrometry using an Ultraflex II MALDI-ToF-ToF mass spectrometer (Bruker Daltonics). The identity of the protein was confirmed by comparing the peptide mass fingerprint (PMF) generated to the theoretical peptide mass fingerprint (PMF) of Cy-GALA-1.

Preparation of rCy-GALA-1 antiserum in rabbits

Anti-rCy-GALA-1 antiserum was generated by injecting a rabbit with 50 μg of rCy-GALA-1, in 0.5 mg/ml QuilA/PBS (1 ml total injection). A secondary injection was administered three weeks later, after which a test bleed indicated a specific antibody response to the recombinant antigen. This experiment was performed under the legislation of a UK Home Office Licence.

Immunoblotting

Soluble somatic antigen extracts were prepared from cyathostomin stages (IL3, EL3, DL and LP) and adult worms of other helminth species (*A. perfoliata*, *Strongylus equinus*, *Strongylus edentatus*, *Strongylus vulgaris* and *P. equorum*) as described previously [9]. However, IL3 were disrupted using a Ribolyser Fast Prep FP120 (Thermo Scientific) instead of a glass homogeniser. Proteins were separated on 4-12% polyacrylamide Bis-Tris gels (NuPAGE MES system, Invitrogen) according to the manufacturer's protocol. For immunoblotting, proteins were transferred to nitrocellulose membranes. To assess cross-reactivity, 0.1 μg rCy-GALA-1 was loaded onto lanes of a 15-well 12% NuPAGE gel using SeeBlue Plus2 protein standards (Invitrogen) for molecular weight estimations. In one lane, 0.4 μg was loaded and after electrophoresis was cut from the gel and stained with Coomassie blue. After transfer, the blot was sliced into separate lanes and blocked in TNTT (10mM Tris, 0.5M NaCl, 0.05% Tween-20, 0.01% thimerosal, pH 7.4). Each of the following sera was used, diluted 1:200 in TNTT: CI and HF sera pools (described above); a pool of 5 horses found to be cyathostomin-free (CF) from a local abattoir and a pool of 12 horses (from the same abattoir) with mucosal cyathostomin burdens of > 100,000 (endemic infected – EI); horses mono-specifically infected with *S. edentatus* or *S. vulgaris* [20], *P. equorum* or *Strongylus westeri* [11]. Also tested was rabbit antiserum (and pre-immunisation samples) generated to the native 20 kDa cyathostomin complex [11]. Sera were incubated at room temperature for 1.5 h. Washing consisted

of three, 5 min incubations in TNTT. The secondary and tertiary steps were as described for the immunoscreening (above), with the exception that the anti-20 kDa antiserum blots were incubated with goat anti-rabbit Ig:HRP (Dako) at 1:500. The blots were developed as for the library screen.

For detection of Cy-GALA-1 protein in somatic extracts of cyathostomins and other helminth species, somatic extracts (9 µg each antigen) were loaded onto 10-well, 4-12% NuPAGE gels, using 10 ng rCy-GALA-1 for comparison. After transfer to nitrocellulose, periodate treatment of the blots was performed as described previously [9]. The blots were probed with pre-immunisation rabbit serum and anti-rCy-GALA-1 serum at 1:300 in TNTT, followed by goat anti-rabbit(Ig):HRP (Dako) at 1:500, and developed as described above. Three 5 min washes were applied between steps.

Enzyme-linked immunosorbant assay (ELISA)

To test reactivity of experimentally infected pony sera (CI) to rCy-GALA-1 over the course of infection, the following conditions were used. Each well of a Microlon High Binding plate (Greiner Bio-One) was coated with 100 µl of rCy-GALA-1 (1 $\mu g ml^{-1}$ in bicarbonate coating buffer, 0.1 M, pH 9.6) overnight at 4°C. Plates were washed with 0.05 % Tween-20 in PBS (PBST), six times. Block solution (2% soya infant powder (w/v) in PBST) was added, 200 µl per well, and incubated for 1 h at 37°C. Plates were washed six times and CI and HF sera (1:200 in block solution), from weekly time points 2 weeks before infection to 16 weeks PI, added and incubated for 2 h at 37°C. After washing, 100 µl goat anti-equine IgG(T) were added, diluted 1:200 in blocking solution. After 1 h at 37°C and washing, 100 µl rabbit anti-goat(Ig):HRP were added, diluted in block at 1:500, and incubated for 1 h at 37°C. To develop the reaction, Sigma*FAST* OPD tablets (Sigma) were dissolved in H_2O according to the manufacturer's instructions and 100 µl added to each well and incubated for 15 min. Fifty µl of 2.5 M H_2SO_4 were added to stop the reaction and the absorbance read at 490 nm. The same conditions were used to measure the anti-rCy-GALA-1 antiserum response to somatic extracts of cyathostomin stages and other adult helminth species extracts, except that these were coated at 2 $\mu g ml^{-1}$. The antiserum and goat anti-rabbit:HRP were used at 1:500.

Immunolocalisation

Cyathostomin DL were fixed in 10% formal saline and immobilised in a solidified gelatin plug by mixing with molten 5% gelatin/PBS (<30°C) and allowing to set. The plugs were then dehydrated with alcohol and xylene and embedded in paraffin wax. Sections were cut at 3 µm using a microtome and the slides stored at 4°C. Immunolocalisation was performed using an EnVision+ System–HRP for rabbit primary antibodies (DakoCytomation) in a Sequenza Slide Rack (Thermo Scientific) at room temperature. After de-waxing, the slides were incubated in 0.5% Tween-80/PBS (PBST80) with 0.3% H_2O_2 for 20 min, to inactivate endogenous peroxidises. Blocking was performed using 100 µl 25% normal goat serum (NGS) in PBST80 for 1 h. Rabbit antisera obtained prior to and after two immunisations with rCy-GALA-1 were diluted 1:100 in 10% NGS/PBST80, and 100 µl incubated on the slides for 1 h. After two washes in PBS at room temperature, 100 µl of HRP-labelled polymer conjugated to goat anti-rabbit Ig, was incubated (neat) for 30 min. The reactions were developed in neat 3-amino-9-ethylcarbazole substrate chromogen for 7.5 min. Slides were washed in H_2O and counterstained using haematoxylin.

Single worm PCR to identify the gene encoding GALA in different cyathostomin species

Genomic DNA was isolated from 54 individually identified adult cyathostomins using the DNeasy Blood and Tissue kit (Qiagen) according to their protocol, but with the addition of a homogenisation step before the proteinase K digestion step; each individual was disrupted briefly using a 1.5 ml microfuge tube homogeniser in 50 µl ATL buffer supplied with the kit. The following 10 species were examined (NB: numbers of worms used for each species is shown in parenthesis): *Cyathostomum catinatum* (10), *Cylicostephanus goldi* (8), *Coronocyclus coronatus* (6), *Cyathostomum pateratum* (6), *Cylicocyclus nassatus* (6), *Cylicostephanus longibursatus* (5), *Cylicocyclus ashworthi* (4), *Cylicocyclus leptostomum* (3), *Coronocyclus labiatus* (1) and *Cylicostephanus minutus* (1). The same primers used in Section 2.3 for RT-PCR were used to amplify a conserved fragment of *Cy-gala* in each species. The cycling conditions were: 2 min at 94°C, followed by 40 cycles of 15 sec at 94°C, 30 sec at 58°C and 60 sec at 72°C, and a final extension at 72°C for 7 min. PCR products were analysed on agarose gels as described above and PCR products from each of the 54 individuals cloned into pGEMT-Easy (Promega)

according to manufacturer's instructions. Each clone was sequenced in forward and reverse directions with vector-specific primers using the commercial sequence facility described above.

RESULTS

Immunoscreening of the cyathostomin DL cDNA library and sequence analysis of Cy-gala-1

The primary immunoscreening yielded 33 positive clones; five of which were excluded as false positives on the basis of the secondary screen using HF sera. The remaining 28 clones contained inserts ranging in size from approximately 500 to 1500 bp. Sequence analysis indicated that 15 of these showed high identity to one another (73-100% at the amino acid [aa] level). One of these (*Cy-gala-1*) represented a full-length coding sequence: i.e. it contained a putative initiation codon, signal peptide and termination codon upstream of a poly-A tail. The entire coding sequence was 223 aa which, after cleavage of the signal peptide, would result in a 206 aa mature protein estimated at 25.6 kDa. Cy-GALA-1 contains a highly conserved domain as revealed by a domain search via BLASTp analysis [28]. The function of this domain is unknown and in *Caenorhabditis elegans* is designated Domain of Unknown Function 148 (DUF148). The Cy-GALA-1 sequence displayed highest aa identity to a sequence from *Nippostrongylus brasiliensis* (accession number: BAB68205; 35% identity over 128 residues), also identified via immunoscreening [38]. Two predicted proteins from *C. elegans* showed 34% identity over 105 residues to Cy-GALA-1. These proteins were 44.5% identical to each other. Also identified, were two trichostrongyloid ESTs: one from *Teladorsagia circumcincta* L3 (accession number: AAM45145), which displayed 32% identity to Cy-GALA-1 over 102 aa, and one from *Ostertagia osteragi* adult worms (accession number: CAD22110) with showed 32% identity over 140 aa. The two *C. elegans* orthologues (referred to here as Ce-KLP-1 (NP_502026) and Ce-KLP-2 (NP_501448)) contain glycine-rich domains which gives them homology to keratin sequences and hence their designation as 'keratin-like' proteins (KLP). All the parasitic nematode sequences described here lack this glycine rich sequence, despite some being previously designated as 'KLP-like' proteins [38]. Rather than classifying Cy-GALA-1 as a KLP, it was instead named to reflect its localisation to the gut (see below). An alignment of Cy-GALA-1 with its orthologous sequences in *N. brasiliensis* and *C. elegans* is depicted in Fig. 1. In all the parasitic nematode

sequences, except that of *T. circumcincta*, a histidine-rich motif precedes DUF148 (Fig. 1); its function is unknown. In addition, four potential N-linked glycosylation sites were identified. Searching Cy-GALA-1 at Nembase gave additional significant hits. All of these EST sequences contained regions with high identity to DUF148 and some had glycine-rich regions. The closest matches were to sequences identified in adult *Ancylostoma ceylanicum* (accession numbers: CB176510, CB190303 and CB339159), with 45-46% aa identity to Cy-GALA-1 over 110 residues.

Temporal transcription pattern of Cy-gala-1

Cy-gala-1 transcript was detected in DL and EL3 cDNA and not in cDNA from LP parasites (Fig. 2). After 40 cycles, similar levels of *coxI* PCR product were observed in DL and LP cDNA. However, a *coxI* PCR product from EL3 was less intense, indicating low quality of EL3 cDNA. This was due to degradation of EL3 RNA caused by the extensive digestion method required to harvest these larvae. These results indicate the apparent specificity of this transcript for mucosal stages; hence the gene was selected for expression of recombinant protein for assessment as a diagnostic marker.

Expression of rCy-GALA-1 and its immunoreactivity

rCy-GALA-1 was obtained from the soluble fraction of the *E. coli* lysate; the purified protein was approximately 28 kDa (Fig. 3). The identity of this protein as rCy-GALA-1 was confirmed by MALDI-ToF-ToF (data not shown). Its molecular weight was slightly higher than the expected size of native Cy-GALA, calculated to be 25.6 kDa, and was due to addition of the His-tag and *E. coli* signal peptide. Anti-rCy-GALA-1 antiserum predominantly recognised the expected size band in somatic DL extracts (Section 3.4 and Fig. 4). The immunoreactivity of the recombinant antigen is shown in Fig. 3. Only IgG(T) in CI and EI sera equine sera bound rCy-GALA-1, indicating that both experimentally and naturally infected horses recognise this antigen. Sera from horses harbouring other parasitic helminths did not contain IgG(T) that bound Cy-GALA-1. The rabbit antiserum to the cyathostomin larval anti-20 kDa complex generated previously [11], showed strong reactivity to rCy-GALA-1.

Levels of rCy-GALA-1-specific IgG(T) in sera from infected *vs.* non-infected ponies [29] were measured by ELISA (Fig. 3). Increases in rCy-GALA-1-specific IgG(T) levels were observed in all infected ponies by 6 weeks PI. A more rapid increase was observed in pony 104. Antigen-specific IgG(T) levels plateaued at 8

weeks PI for 104 and 12 weeks PI for 101 and 105; these levels remained elevated until the end of the measurement period at 16 weeks PI. No significant increases in rCy-GALA-1-specific IgG(T) levels were observed in any of the HF ponies throughout the experiment. Murphy and Love (1997) [29] described clinical signs in the infected animals from 4-6 weeks PI. While all showed a slower increase in percentage weight gain than the control group, pony 104 showed a drop in weight gain over weeks 4-8 PI. These signs may indicate a higher level of infection in 104.

Anti-rCy-GALA-1 antiserum reactivity was tested against somatic extracts from *A. perfoliata*, *P. equorum*, *S. edentatus*, *S. vulgaris* and *S. equorum* [Fig. 4]. No reactivity was observed except to a band at 38 kDa in the *P. equorum* extract. Binding to this band was less than that seen in the cyathostomin DL lane (Fig. 4).

Detection of Cy-GALA-1 in different cyathostomin stages

Antiserum raised to rCy-GALA-1 was used to investigate the presence of the native protein in different cyathostomin stages (Fig. 4). This antiserum bound the 28 kDa recombinant antigen (Fig. 4, lane 1): an additional band at 53 kDa was bound and may represent a dimeric form of Cy-GALA-1. The anti-rCy-GALA-1 antisera showed reactivity to EL3 and DL somatic extracts but not to adult extract (Fig. 4). Immunoreactivity to antigens in EL3 and DL stages was primarily directed at molecules of approximately 26 kDa, corresponding to the calculated molecular mass of Cy-GALA-1. Two other EL3 and DL antigens were bound by IgG in anti-Cy-GALA-1 antisera, one at approximately 45 kDa and the other at 55 kDa. The ELISA results indicated high reactivity to DL, however no binding was observed in EL3 or adult extract.

Immunolocalisation of Cy-GALA-1

DL were subjected to immunolocalisation studies (Fig. 5). Reactivity was detected in the gut of individual worms, where considerable staining was observed on the gut epithelium and in the gut lumen. No reactivity was detected to any other structures in the nematodes.

Single worm PCR to identify the gene encoding Cy-GALA in different cyathostomin species

Single worm PCR experiments were performed using primers to amplify a 220 bp fragment of *Cy-gala-1* from 50 morphologically-identified adult worms

encompassing 10 species. A PCR product was obtained from all nematodes tested and sequencing confirmed that PCR products representative of each species encoded *Cy-gala* sequence. Fig. 6 shows a schematic representation of this fragment and PCR products from each species. There was variation in size of the PCR product obtained from different species; from 267 bp (for all *C. coronatus* individuals) to 284 bp (for one *C. goldi* individual). This variation was due to a difference in intron size at this site amongst the species. The precise location of the intron was conserved as indicated by splice site analysis (Fig. 6). Nucleotide identities between individuals from different species ranged from 78.9-99.1% for the whole fragment. Higher nucleotide identities were observed in the coding region; interspecies variation ranged from 82.2-98.9% over 180 nt, while the amino acid identities were 80-100% over 60 residues. At the aa level, intra-species variation was as follows;: *C. catinatum* 93.3-100%; *Cs. goldi* 90.0-100.0%; *Co. coronatus* 96.7-100.0%; *C. pateratum* 93.3-100%; *Cc. nassatus* 88.3-98.3%; *Cs. longibursatus* 91.7-100%; *Cc. ashworthi* 90.0-100.0%; *Cc. leptostomum* 88.3-100.0%. In an attempt to assign a species for the library clone, *Cy-gala-1*, the coding sequence from each individual was compared against *Cy-gala-1* in this 220 bp fragment. The highest identity was found to a *C. pateratum* individual (97.8% nt identity and 98.3% aa identity). Therefore, with the available sequence data for each species, we have provisionally identified *Cy-gala-1* as belonging to *C. pateratum*.

Optimisation of antigen cocktails.

The optimum concentration of antigen to use in an ELISA using a cocktail of antigens was evaluated using sera from cyathostomin infected (positive) and non-infected (negative) animals. Figure 7A shows the serum antibody response to varying concentrations of antigen in two different cocktails of antigen (CT1 and CT2). CT1 contains GALA-1, -Gala 2 and -3. CT2 contains these three antigens plus CID1. Individual antigen concentration is shown on the x-axis and optical density (O.D) on the y-axis. Figure 7B shows the ratio of the OD signal obtained om cyathostomin infected (positive) *vs.* uninfected (negative) animals in an ELISA. Individual antigen concentration is on the x-axis and ratio of positive to negative optical density on the y-axis.

Evaluation of antigen cocktail for discriminating different levels of infection.

Two different cocktails of antigen were tested in an ELISA to assess their potential for discriminating different levels of mucosal infection. Figure 7 C and D

shows mean serum antibody response to cocktail 1 (CT1) and cocktail 2 (CT2) respectively in groups of horses with varying infection levels. CT1 and CT2 were as described above. Horses were grouped as follows according to total mucosal parasite burden (TMB). Neg; uninfected horses TMB = 0 (n=5),Low; TMB = 0-20000, (n=8), Medium; TMB=20000-100000, (n=7), High; TMB = >100000 (n=26). Error bars show +/- standard error of the mean. O.D = optical density.

DISCUSSION

Identification of the cyathostomin GALA sequence is an advance in the development of an ELISA for the diagnosis of larval cyathostominosis. Three important criteria were met by this protein: 1) it appeared to be specific to larval stages; 2) there was no cross reactivity with the other equine helminth species assessed here and 3) the gene encoding the protein was isolated from all cyathostomin species examined with a relatively low level of sequence variation amongst the species. Furthermore, serum IgG(T) responses to rCy-GALA-1 increased within 5 weeks of the administration of an experimental infection and the protein was also the target of IgG(T) responses in naturally infected horses.

The RT-PCR, immunoblot and ELISA results indicated that Cy-GALA-1 is restricted to parasitic larval stages, particularly DL stages. This is a vital feature for a diagnostic marker that specifically indicates mucosal larval burden. Despite numerous attempts, RNA extracted from EL3 was of relatively poor quality so it was difficult to judge precise levels of transcription in these stages. EL3 require extensive digestion in pepsin/HCl at 37°C to remove them in sufficient quantity from the intestinal mucosa and submucosa and so it is technically difficult to obtain sufficient high quality RNA. The EL3 somatic protein extracts also contained a small amount of contaminating host protein (it is impossible to totally separate every single worm from its host capsule), and this may have resulted in the lower levels of reactivity of EL3 extracts to Cy-GALA-1 antiserum as indicated by the ELISA results. Immunolocalisation was also attempted in EL3, but degradation resulted in a lack of distinct morphology and no specific binding was observed (data not shown). Therefore it remains to be fully elucidated if Cy-GALA is a significant immunogen of EL3, or is predominantly an antigen of the later larval stages. Immunolocalisation studies of diseased equine mucosa are planned, to provide EL3 embedded in their mucosal cysts.

Serum IgG(T) responses to rCy-GALA-1 over the time course of an experimental infection showed that the antigen is a reasonably early indicator of infection and these responses were identified whilst the infections were not patent [29]. Indeed, in these ponies, the infections never progressed to patency even though the experiment was continued until 60 and 62 weeks PI in two of the animals. Substantial increases in reactivity were observed at 5 weeks PI in one animal (pony 104) and by 6 weeks in all ponies. cyathostomin larval-specific serum IgG(T) responses were analysed previously in these animals and similar dynamics of responses were observed to the 20 and 25 kDa complexes purified from EL3/DL mixtures [11]. Furthermore, serum IgG(T) reactivity to crude larval antigen was also observed to increase only after 6 weeks PI in these ponies [9], suggesting that only by this time point do larvae stimulate a detectable serum IgG(T) response. Pony 104 had the most pronounced increase in IgG(T) to rCy-GALA-1 and this is similar to its response to crude larval antigen and the purified 20- and 25-kDa antigen complexes [9, 11]. The clinical signs observed in this pony (reduced weight gain, lowest plasma fructosamine) indicate that it may have had a greater burden of mucosal larvae [29]. Indeed, when this animal was euthanized at 20 weeks PI it was found to have a high cyathostomin burden. Unfortunately the other two ponies in the group were necropsied at 60 and 62 weeks PI so their burdens cannot be directly compared with pony 104. Nevertheless, the data provides preliminary evidence that this recombinant antigen may be able to distinguish varying degrees of disease.

As mentioned above, there is similarity of the IgG(T) response to rCy-GALA-1 and to the two larval antigen complexes purified and shown to have diagnostic potential previously [10, 11]. The molecular mass of Cy-GALA, estimated at 25.6 kDa, means that it could feasibly be a component of the 25 kDa antigen complex, an observation supported by the results using anti-rCy-GALA-1 against EL and DL somatic extracts in western blots. Antiserum generated to the 20 kDa complex in rabbits also bound rCy-GALA-1 indicating its presence in this complex also. This is not altogether surprising as these complexes were excised rather crudely from SDS-polyacrylamide gels [10, 11].

Specificity of Cy-GALA-1 in the cyathostominae was confirmed by probing the recombinant protein with sera from horses infected mono-specifically with heterologous helminth species. While experimentally infected (CI) and naturally infected (EI) horses recognised rCy-GALA-1, IgG(T) in serum from horses with large

strongyle infections (*S. edentatus, S. westeri* or *S. vulgaris*) and *P. equorum* infection, did not bind the antigen. Cross-reactivity was further explored by probing somatic extracts of other equine parasites with anti-rCy-GALA-1 serum: extracts from *A. perfoliata*, *P. equorum*, *S. edentatus*, *S. vulgaris* and *S. equorum* were analysed. In the ELISA no binding above background levels was observed in any of the five other parasite extracts. In the immunoblot, there was a degree of binding to a band of approximately 38 kDa in the *P. equorum* extract, but this was of far less intensity than binding observed in the cyathostomin DL samples. Furthermore, there was no cross reactivity to *P. equorum* antigens when the samples were assessed using the ELISA.

The presence of sequences encoding GALA-like proteins was confirmed in 10 cyathostomin species, indicating ubiquity of this gene in the group. There are currently 50 recognised cyathostomin species [23], and while a large number of species are often found in infected individuals [6, 7], the bulk of the burden is consistently found to comprise 5-10 species [26, 27, 36]. Nine of the species explored in this study belong to the 10 most common cyathostomins as identified by Reinemeyer et al. (1984) [36], Ogbourne (1976) [30] and Lictenfels et al 2001 [22]. The presence of Cy-GALA in these species indicates it is likely to be present in most, if not all, cyathostomins. An analysis of the sequence of *Cy-gala-1* amongst the cyathostomins indicated a low level of sequence diversity across the selected 220 bp region. It is possible that greater diversity exists outside this region and the full-length cDNA sequences of *Cy-gala* are currently being isolated from a number of species to investigate this further. Promisingly, for development of a specific immunoassay, the levels of sequence diversity identified thus far are substantially lower among cyathostomins than they are when the *Cy-gala* sequences are compared to orthologous sequences in other nematode species, i.e. 80-100% *vs.* 25-35% identity. The nematodes that were present in the CI pony group unfortunately had not been identified, so it is difficult to compare levels of rCy-GALA-1 IgG(T) with the species present.

A factor that must be considered in the development of any helminth immunodiagnostic assay is the length of time that circulating specific immunoglobulin levels take to return to normal values after anthelmintic treatment. Since the ponies used in the experimental infection were not treated with anthelmintic before necropsy, this could not be assessed here. Studies on a commercially-available serological ELISA for *A. perfoliata* [33, 34], which is based on the specific binding of

IgG(T) to a purified 12/13 kDa antigen complex, indicated that post-treatment IgG(T) levels can take months to reduce to 'non-infection' levels [2, 4]. Also, Kjaer et al. (2007) [18] found that two thirds of horses which had no visible signs of tapeworm infection at necropsy had ELISA ODs higher than the current accepted cut-off for infection (0.2). Despite this, the *A. perfoliata* 12/13 kDa antigen ELISA is still regarded as the most useful diagnostic tool for infection [1, 18]. These observations suggest that circulating IgG(T) levels may remain high for a time after treatment and this will be considered when designing how a cyathostomin diagnostic assay, based on IgG(T), could be used in future.

No function has been ascribed to orthologues of Cy-GALA in other nematode species and only Nb-KLP has been characterised in any detail [38]. It was speculated that Nb-KLP may be a cuticular protein, based on its identity to Ce-KLPs, which are described as 'keratin-like'. However the authors did not explore this further. Ce-KLP-1 and -2 encode hypothetical proteins, and some information regarding these is available in WormBase (www.wormbase.org). Both are predicted to be alpha-helical proteins, and Ce-KLP-1 has been confirmed by transcript evidence, while Ce-KLP-2 has been partially confirmed. Ce-KLP-1 shows no RNAi phenotype, while Ce-KLP-2 displays 'embryonic lethal', indicating that it may play a role in development. An anatomic expression plan is available for Ce-KLP-2, showing expression in pharyngeal muscles and tail neurons which is different to what was observed here with localisation of Cy-GALA to the worm intestinal lumen. The function of this molecule remains to be elucidated.

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Additional immunoreactive clones: The following table lists additional sequences encoding immunoreactive cyathostomin antigens which were identified from the larval cDNA library, from two screenings which revealed distinct clones. The first immunoscreen (A) used serum from experimentally cyathostomin-infected ponies (from a previous study[1]), and the second immunoscreen (B) used a pool of sera from naturally infected horses; both groups had high parasite burdens. The antigens which have been checked for immunogenicity and cross specificity by recombinant bacterial expression are also indicated.

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Antigen	Number of clones per screen		Closest homologues (Accession numbers in brackets)	Amino acid identity	Transcription pattern			Immunogenicity	Cross specificity
	A	B			EL3	DL	LP		
Gut-associated larval antigen (GALA)	15	1	Keratin-like protein, *Nippostrongylus brasiliensis*, (BAB68205).	35% over 128 a.a.	+	++	-	+	+
			Keratin-like proteins, *Caenorhabditis elegans* (NP_502026 and NP_501448)	34% over 104 a.a.					
Glutathione-S-transferase (GST)	1	0	Cytosolic GST from *Oesophagostomum dentatum* (ACA30415)	85% over 209 a.a.	-	+	+		
Galectin-1 (GAL-1)	1	0	Galectin family member, *C. elegans* (NP_495163)	83% over 279 a.a.	-	++	+		
Galectin-2 (GAL-2)	0	1	Galectin family member, Haemonchus contortus (AAF63406)	91% over 259 a.a.	+	++	++		
Nematode polyprotein allergen/antigen (NPA)	4	0	NPA from *Dictyocaulus viviparus* (Q24702)	42% over 314 a.a.	-	++	++		
Cyathostomin immunodominant antigen-1 (CID-1)	3	0	EST from larval-stage *Necator americanus* (BG467549). Function of this is unknown.	59% over 61 a.a.	-	++	++	+	
Surface associated antigen (SAA)	4	8	SAA-2, *N. americanus* (ACE79378)	71% over 146 a.a.	+	++	++		
Fatty acid/retinol binding protein -1 (FAR-1)	0	1	Putative ES protein with FAR binding domain, *Ostertagia ostertagi* (CAD20464)	45% over 100 a.a.	+	++	++		
Fatty acid/retinol binding protein -2 (FAR-2)	0	1	FAR binding protein, *Ancylostoma ceylanicum* (ACC76809)	72% over 160 a.a.	-	++	+		
Globin (GLO)	0	15	Cuticle globin, from *Syngamus trachea* (AAL56426)	54% over 161 a.a	+	++	++		
Clone of unknown function -20a (Unk-20a)	0	1	No homology found	NA	-	++	++		
Unk-46a	0	1	Third-stage larval EST, *N. brasiliensis* (EH359049)	33% over 124 a.a.	-	++	++		
Unk-50a	0	1	Hypothetical protein, *C.elegans* (NP_490737)	33% over 140 a.a.	-	++	+		

Homologues of cyathostomin gut-associated larval antigen (Cy-GALA)

Homologue	Amino acid identity to Cy-GALA-1	Putative species
Cy-GALA-1	-	*C. pateratum*
Cy-GALA-2	83.3 %	*C. nassatus*
Cy-GALA-3	77.7 %	*C. coronatus*
Cy-GALA-4	93.0 %	*C. catinatum*

Sequences of a conserved region of GALA from individual cyathostomin species

Species	Sequence	Number of individuals	Intraspecies aa identity range
C. ashworthi	LTFAEKKGKISEWAKKYNVVDEVASYNAYREKLKQEHRKNVS(E/V)LVSGLP(G/D)AVKKVN(E/V)LLD	4	90.0-100%
C. catinatum	LTFAEKK(E/K)EISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSALPNAVKKVNDLLD	10	91.7-100%
C. coronatus	LTFAEKKEKISEWAKKYKVEDEVASYNAYREKLKQEHRKNVSELVSALPGAVKKVNELLD	6	96.7-100%
C. goldi	LTFAEKKKEISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSDLPSAVKKVNDLLD	8	90.0-100%
C. labiatus	LTFAEKKEKISEWAKKYNVVDEVARYNAYREKLKQEYRKNVSELVSGLPNAVKKVNDLLD	1	-
C. leptostomum	LTFAEKKGKISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSGLPGAVKKVNELLD	3	88.3-100%
C. longibursatus	LTFAEKKEEISKWAKKYNVVDEVASYNAYREKLKQEHRKNVSEIVSDLPNAVKKVNDLLD	5	91.7-100%
C. minutus	LTFAEKKEKISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSQLVSALPNAVKKVNDLLD	1	-
C. nassatus	LTFAEKKEKIGEWAKKYNVVDEVAXYNAYREKLKQEHRKNVSELVSGLPNAVKKVNELLD	6	88.3-100%
C. pateratum	LTFAEKK(K/E)EISEWAKKYNVVDEVASYNAYREKLKQEHRKNVSELVSALPNAVKKVNDLLD	6	93.3-100%

Claims

1. A method of diagnosing a cyathostomin infection in an animal, said method comprising the step of identifying a level of anti-cyathostomin larval antigen antibodies in a sample, wherein a level of anti-cyathostomin larval antigen antibodies is indicative of a cyathostomin infection.

2. A method of diagnosing a cyathostomin infection in an animal, said method comprising the step of identifying a level of cyathostomin larval antigen in a sample, wherein a level of cyathostomin larval antigen is indicative of a cyathostomin infection.

3. A method for determining whether or not an equine subject should be treated with anthelmintic drug, said method comprising the step of identifying a level of anti-cyathostomin larval antigen antibodies in a sample according to claim 1 and/or identifying a level of cyathostomin larval antigen in a sample, wherein a level of anti-cyathostomin larval antigen antibodies and/or cyathostomin larval antigen, is indicative of an equine subject that should be administered an anthelminitc drug.

4. The method of any preceding claim, wherein the level of anti-cyathostomin larval antigen antibodies or cyathostomin larval antigen may be evaluated relative to the level of anti-cyathostomin larval antigen antibodies or cyathostomin larval antigen present in a reference or control sample derived from a healthy animal, an animal not having a moderate or high mucosal burden of cyathostomin parasites and/or an animal not having larval cyathostominosis.

5. The method of claim 1, 3 or 4, wherein the sample is contacted with one or more cyathostomin larval antigen(s), under conditions which permit binding with any anti-cyathostomin larval antibodies present in the sample.

6. The method of claim 5, wherein the sample is contacted with one or more cyathostomin larval antigen(s) comprising, or encoded by, a sequence selected from the group consisting of those provided by SEQ ID NOS: 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 and any fragments, mutants or variants thereof.

7. The method of claim 5, wherein the sample is contacted with an antigen comprising or encoded by the sequences of SEQ ID NOS: 1 or 2 and any fragments, mutants or variants thereof, and optionally, one or more other antigens comprising, or encoded by a sequence selected from the group consisting of those provided by SEQ ID NOS: 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 and any fragments, mutants or variants thereof.

8. The method of claim 5-7, wherein the cyathostomin larval antigen(s) are bound, conjugated or immobilised on, or to, a suitable substrate.

9. The method of claim 8, wherein the substrate comprises glass, nitrocellulose, paper, agarose and/or a plastic material.

10. The method of claims 8 or 9, wherein the substrate takes the form of a microtitre plate.

11. The method of claim 2, wherein the sample is contacted with one or more cyathostomin larval antigen binding agent(s), under conditions which permit binding with any cyathostomin larval antigens present in the sample.

12. The method of claim 11, wherein the sample is contacted with one or more agent(s) capable of binding cyathostomin larval antigen(s) comprising, or encoded by, a sequence selected from the group consisting of those provided by SEQ ID NOS: 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 and any fragments, mutants or variants thereof.

13. The method of claim 11, wherein the sample is contacted with a binding agent capable of binding an antigen comprising or encoded by the sequences of SEQ ID NOS: 1 or 2 and any fragments, mutants or variants thereof, and optionally, one or more other binding agents capable of binding antigens comprising, or encoded by a sequence selected from the group consisting of those provided by SEQ ID NOS: 3 ,4,

5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 and any fragments, mutants or variants thereof.

14. The method of claims 11-13, wherein the binding agent(s) are bound, conjugated or immobilised on, or to, a suitable substrate.

15. The method of any preceding claim, wherein the animal is a member of the Equidae family.

16. The method of any preceding claim, wherein the animal is a horse.

17. The method of any preceding claim, wherein the sample is a biological sample selected from the group consisting of: whole blood; serum; plasma; saliva; sweat; semen; tissue biopsy; tissue scraping; tissue/organ wash/lavage; and faecal preparations.

18. The method of any preceding claim, wherein a level of anti-cyathostomin larval antigen antibody in a sample is identified using an immunological detection technique.

19. The method of claim 18, wherein the immunological detection technique is selected from the group consisting of Western blot; dot blot and enzyme-linked immunosorbent assays (ELISA).

20. A substantially purified or recombinant cyathostomin protein comprising an amino acid sequence at least 60% homologous or identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1; 3; 5; 7; 23; 29 and 31.

21. A substantially purified or recombinant nucleic acid molecule that encodes a protein according to claim 20.

22. An substantially purified or recombinant nucleic acid at least 60% homologous or identical to a nucleic acid selected from the group consisting of SEQ

ID NOS: 2; 4; 6; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 24; 25; 26; 27; 28; 30; 32; 33 and 34.

23. A vector comprising a nucleic acid according to claim 21 or 22.

24. A host cell transformed with the vector of claim 23.

25. A kit for diagnosing cyathostomin infections, said kit comprising components selected from the group consisting of:

(i) a substrates, optionally having one or more cyathostomin larval antigens bound or immobilised thereto;

(ii) cyathostomin antigen preparation(s);

(iii) agents capable of binding cyathostomin larval antigens;

(iv) one or more oligonucleotide(s)/primer(s) for detecting/amplifying/probing cyathostomin larval antigen encoding sequences; and

(v) reagents to facilitate, immunological detection assays, sequencing, PCR and/or RFLP analysis.

(vi) instructions for use.

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Cy-GALA-1       ---MNKTLTFLTVVSAVALAQGVMDLFGEEGRE-----------EHRRHHRHSLLPPYL 45
Nb-KLP          ---------MKALLIAVLALTAAAHYRGKESET-----------GHGRHHHHPPPPPFL 39
Tc-KLP          MKLLLLSIFLVACVLSVDGWNRVRALTKGEERTTDDESDSNCCPCHHHCHYHHHKMPPFL 60
Oo-KLP          ------KSAXFLLLIGATVLGHARHNEGCPREE-----------SHR----AQPRPKFL 38
Ce-KLP-1        -MALSYSFIFTLFAFSAVVLAGPGGRHGHGGGG-----------FGG----APQLPPFL 43
Ce-KLP-2        MSYYSTSLYIFAITMATMVLAGPRGGFGGGPGG-----------PGGRGRHGPPMPPFL 48
                                                            * :*

Cy-GALA-1       HNVSCEAKWEYFKIVGNRSLTFAEKRKEISEWAKKYN--VVDEVASYNAYREKLKQEHRK 103
Nb-KLP          KDVDKSARKEFFAIVKNKTLTIAEQKAAVLEWAECHG--IKDEVEQFQQKMASLGDEIKK 97
Tc-KLP          KHVSADARWEYYAIIRDMFSSMSEKLKKLDEWAKKQDPEVKKGMEAYFKNIDMYWKDVNK 120
Oo-KLP          HHVGIKARREYFHIVRS-GEIIAKQDEQILDWAKKYG--VEEEVEEFNNKTASYVEELVQ 95
Ce-KLP-1        QNVTAEGRQAFFAIVSNTSLTISETESQISSWAQTYG--VSSQVTEFQTKVEEKLNEIKQ 101
Ce-KLP-2        QNVTDEGRRAFFDIARNQNLTIAEMESQTSTWAQTYG--VSDVYSEFEANITAHRNEVQQ 106
                :.*  ..:  :: *  .   :::       **:  .  : .   :        .:  :

Cy-GALA-1       NVSELVSALPNAVKKVNDLLDNENQTPRQLYVALRKLGRQNPALYRIVEYTNVAVR---- 159
Nb-KLP          NVAELISKLPAAFQSFSAVMESENQTRREQKDRLKALKDEQPKVFNVLKAAFHQFKP--- 150
Tc-KLP          NMTMTLEELPKIYPKVYEIMADLDLTPREIYKKIRDLQMSKMTSHSLYAVAMAVIHTGG- 173
Oo-KLP          NVTNLIAELPTALEAFLNITQNKDQTRMEMKKALREMRTEEFEVFDALKAAFKVFKPNHC 148
Ce-KLP-1        NVTAVINNLSTVETQLEAIFANKSQTIREQFQALGQLKDQYPQEVGVLLFLAKPKGEHGG 161
Ce-KLP-2        NVTQVVSQLSAAQTALEAVMNNKNQTRQQMKEAIDNLKTQYPQEIPALFFISGSF----- 158
                *::  :  *.     .  :  . . * :    :  :  .

Cy-GALA-1       -LRSEEVDEQEQRRRLSALPFGDHNDNLEEQDFGEQDFRYVYG----------------F 202
Nb-KLP          ------MNEG-----PGKFVGGRRHRRQAQEDCPEAIFLFEID---------------- 186
Tc-KLP          ----------------AEYPYLMDNDMFFETLATPKIRNLFN---------------- 205
Oo-KLP          ---------LYHRCTDSQSSEELVDDWMDFQEKDDQISKMLDP---------------- 189
Ce-KLP-1        QGPFGGFPGGHQGGFPGGNQGGFGGNQGGFGGNQGGFPFGNQG-GNQGGFPFGNPGNQGG 220
Ce-KLP-2        ----RRGPGGRHGG-PGGPGGRRMGPGGRGGDSREGPMMGGMGRGGFGGQGMGGMG--AG 214

Cy-GALA-1       ECARFLLQNGRMFGLNTDERY 223
Nb-KLP          ----ENEEEKPTPKPKRRNRF 203
Tc-KLP          -------NRNTCNN------- 212
Oo-KLP          ------HDEFTMMQKSGV--- 201
Ce-KLP-1        FGGNQGGNQGGFGGNRGGRGF 241
Ce-KLP-2        LGQGRRGGPDSMNESSDVNDF 235
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Figure 1

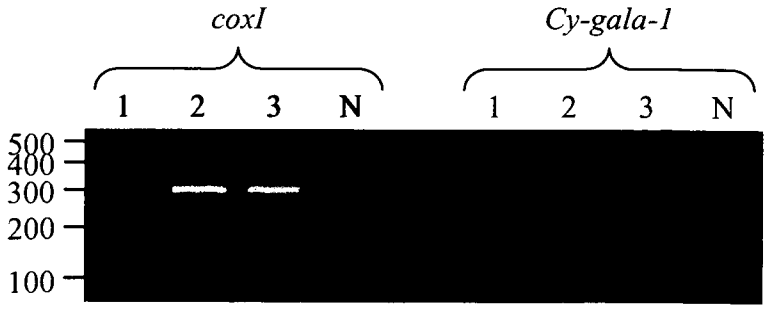


Figure 2

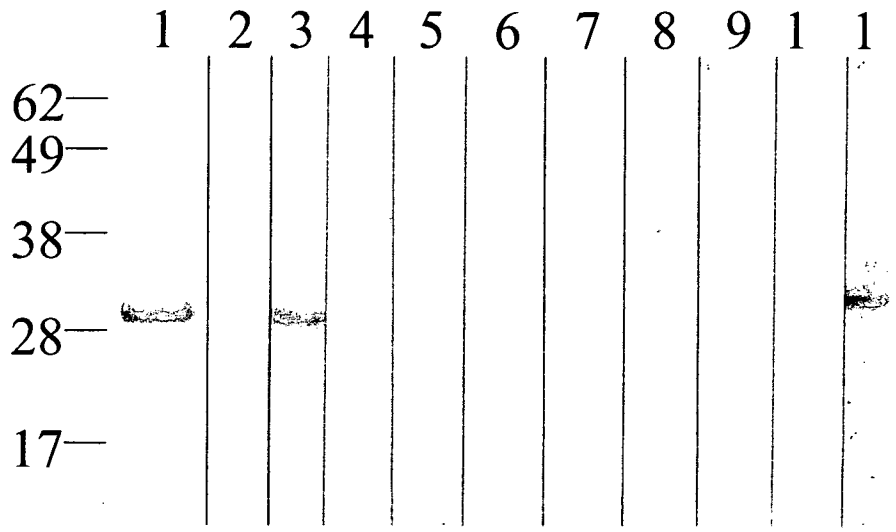


Figure 3A

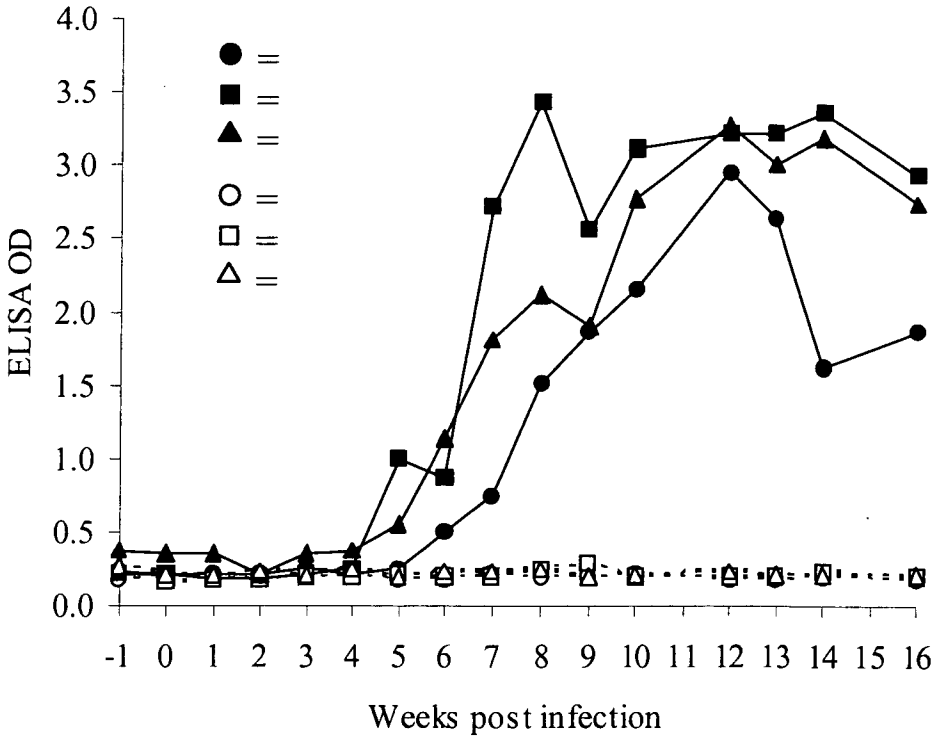
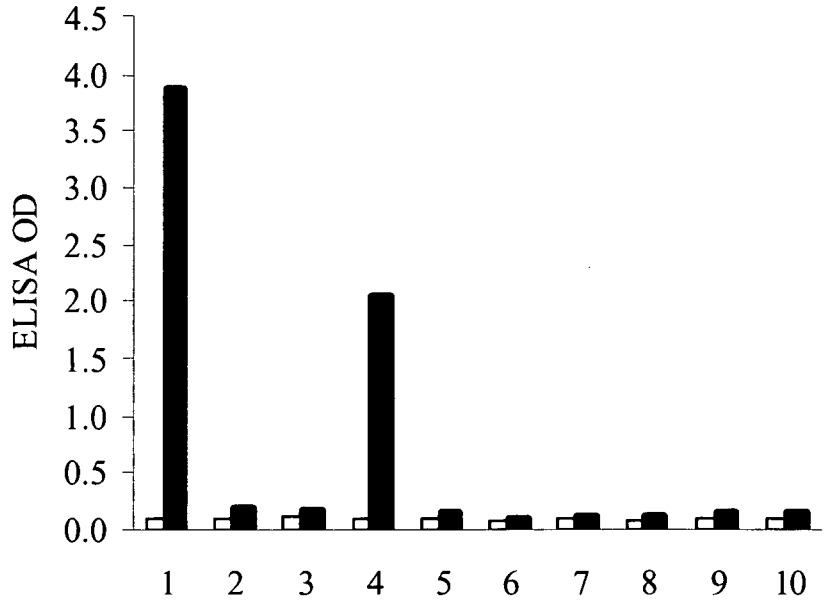


Figure 3B



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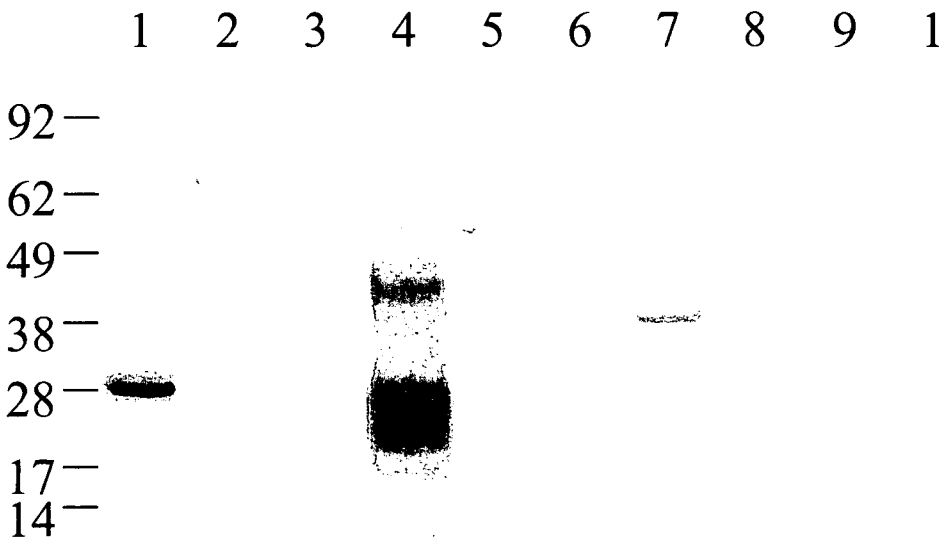


Figure 4B

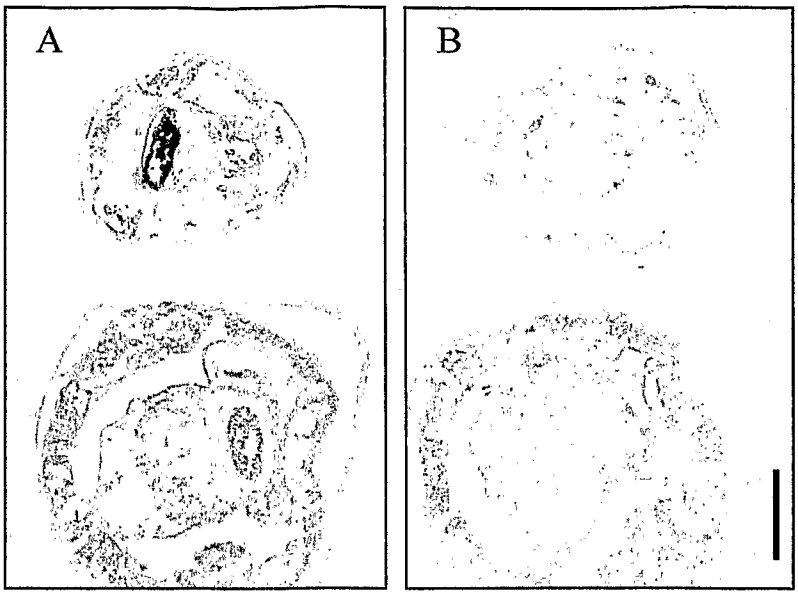


Figure 5

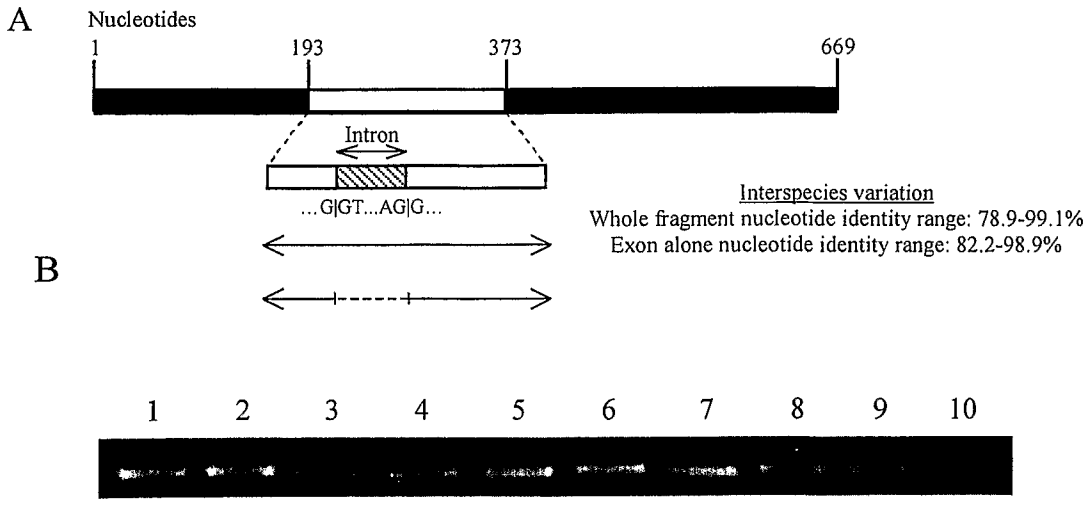
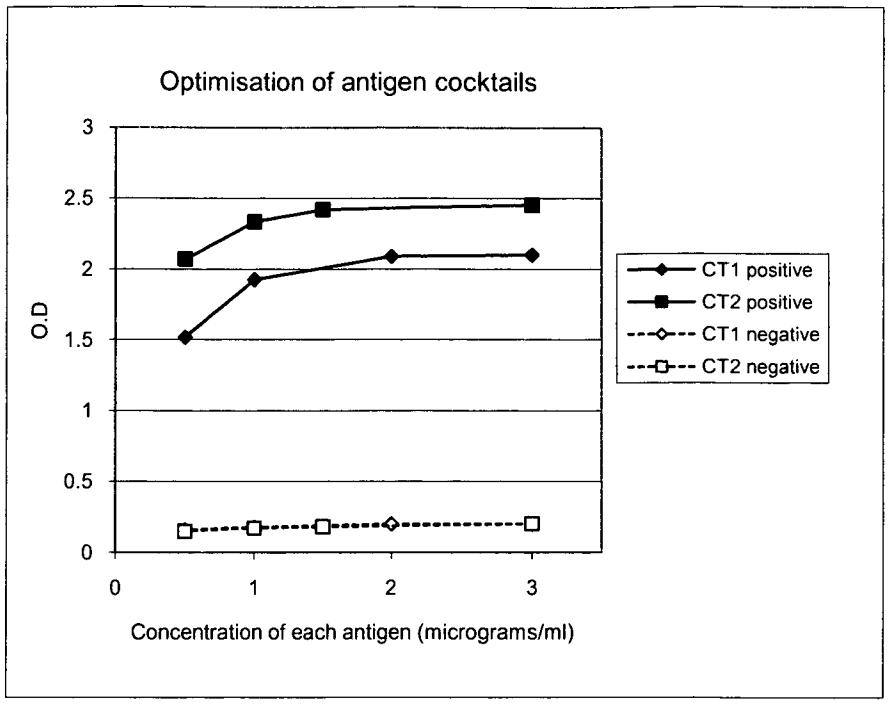
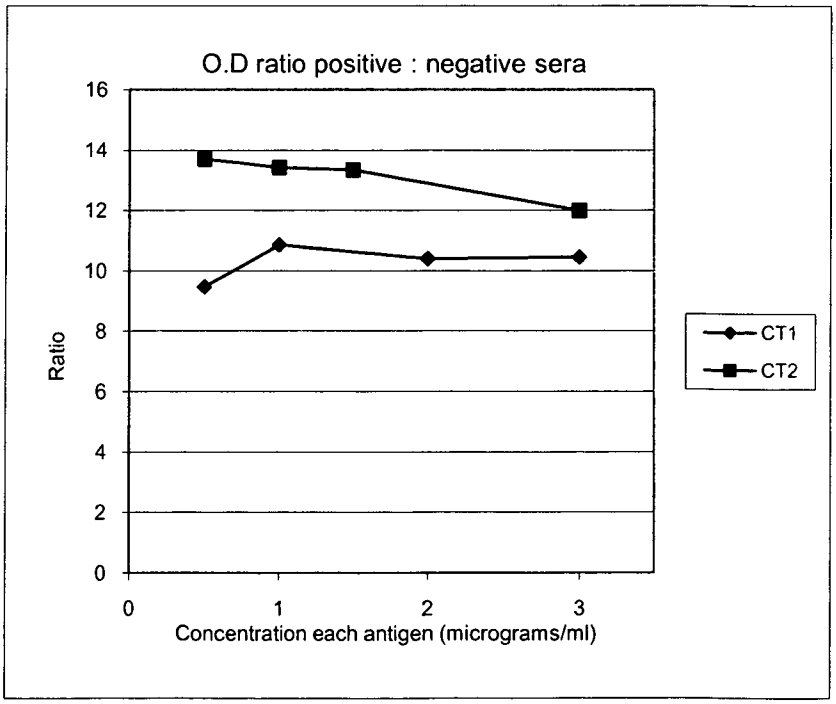


Figure 6

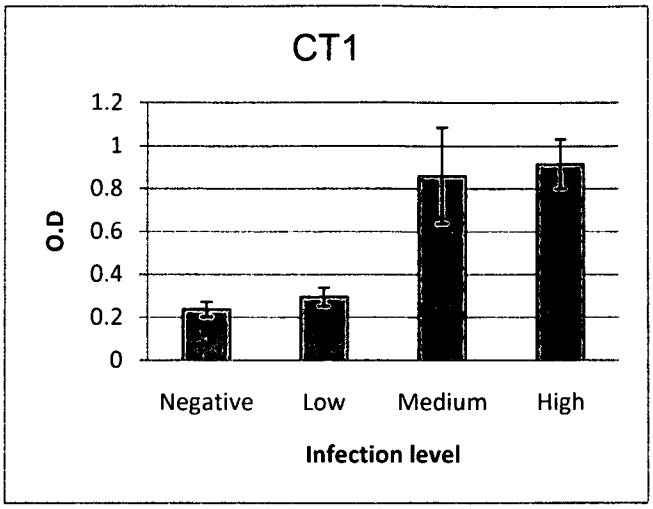


A



B

Figure 7 A & B



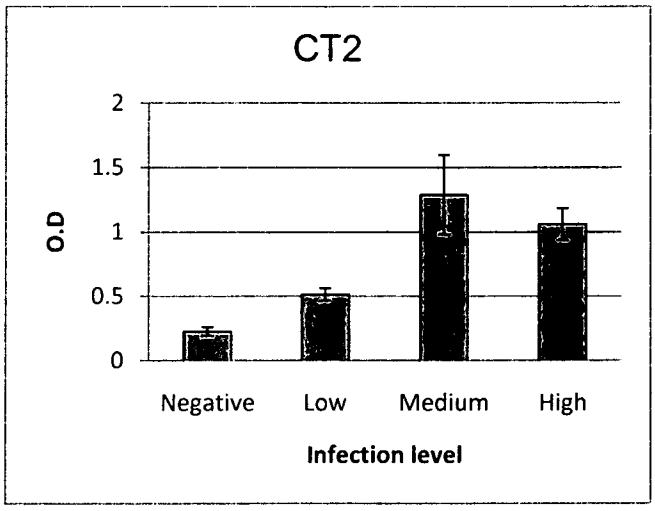
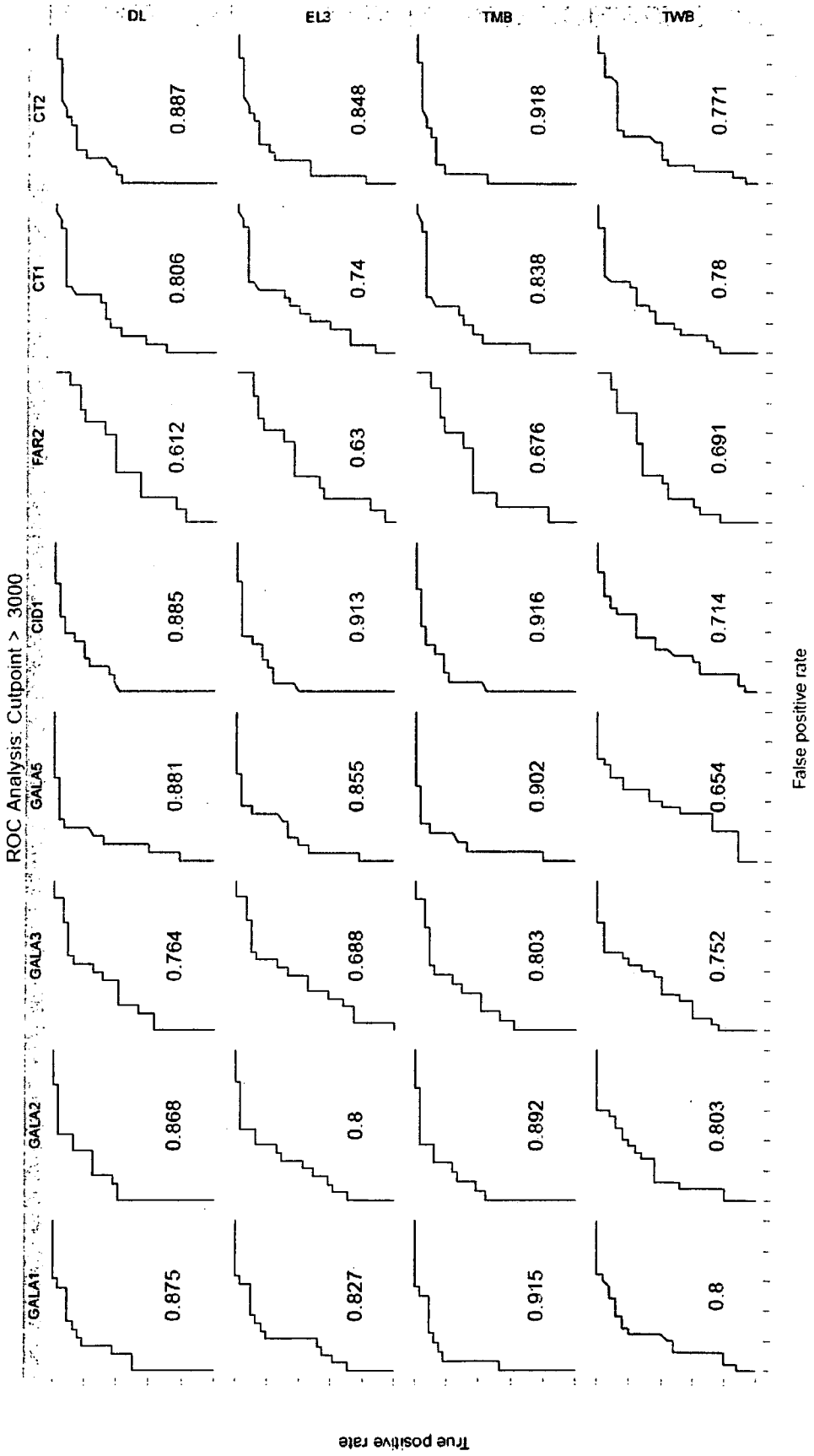


Figure 7 C and D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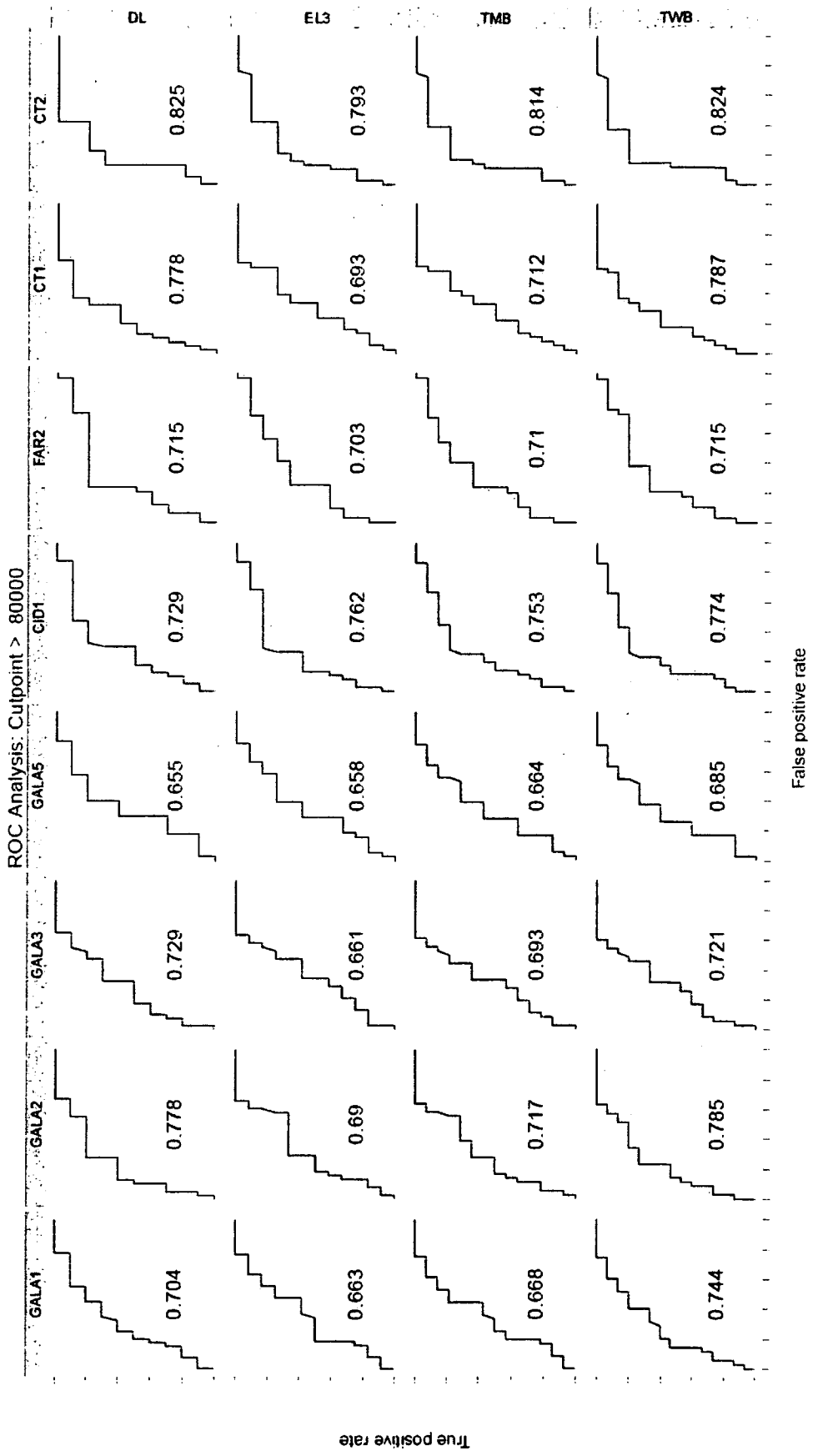
TWB
CT2
CT1
FAR2
CID1
GALA5
GALA3
GALA2
GALA1
0.887
0.848
0.918
0.771
0.806
0.74
0.838
0.78
0.612
0.63
0.676
0.691
0.885
0.913
0.916
0.714
0.881
0.855
0.902
0.654
0.764
0.688
0.803
0.752
0.868
0.8
0.892
0.803
0.875
0.827
0.915
0.8
False positive rate
True positive rate

Figure 8A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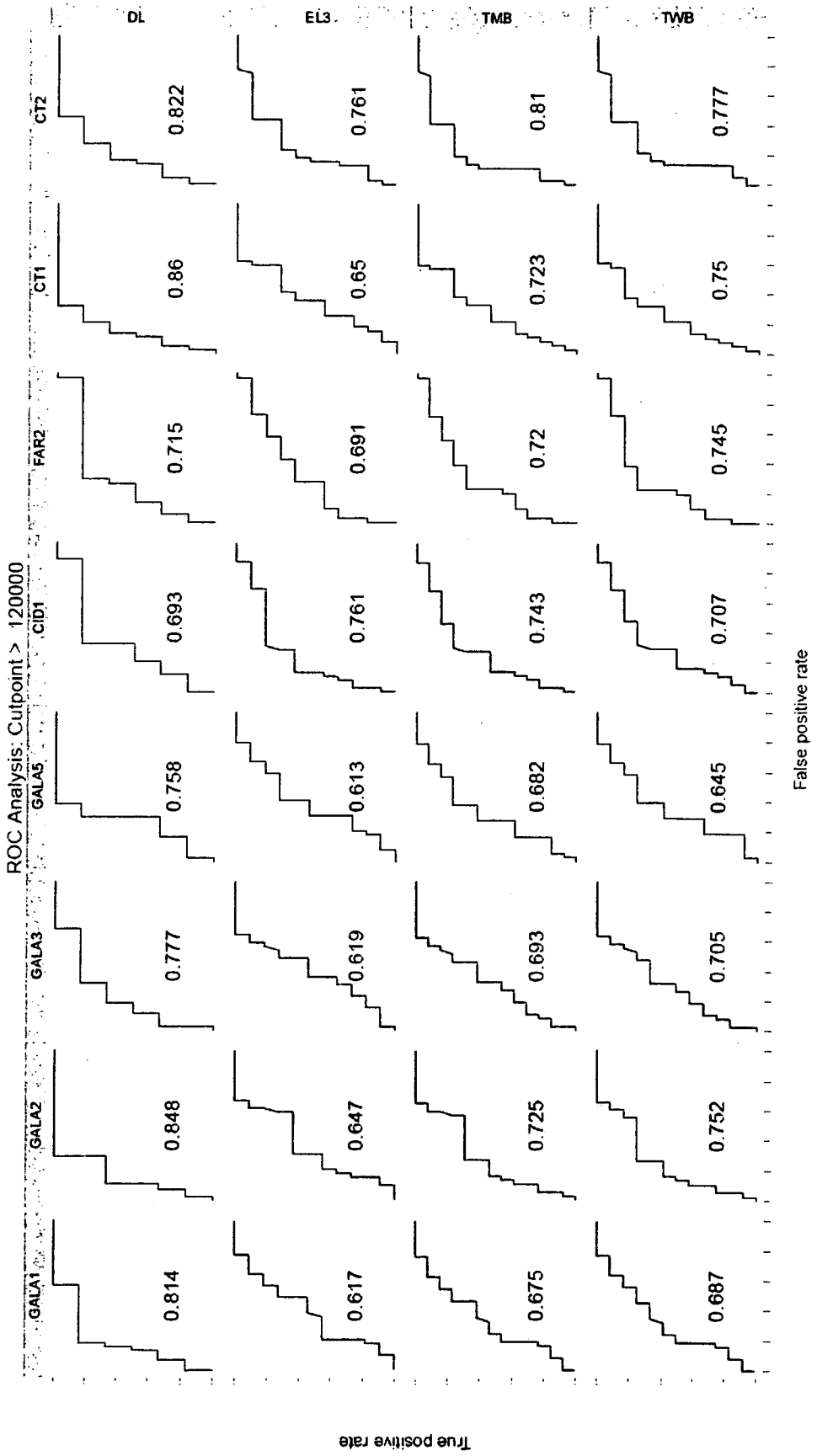
TMB
TWB
CT2
CT1
FAR2
CID1
GALA5
GALA3
GALA2
GALA1
0.825
0.793
0.814
0.824
0.778
0.693
0.712
0.787
0.715
0.703
0.71
0.715
0.729
0.762
0.753
0.774
0.655
0.658
0.664
0.685
0.729
0.661
0.693
0.721
0.778
0.69
0.717
0.785
0.704
0.663
0.668
0.744
True positive rate
False positive rate

Figure 8B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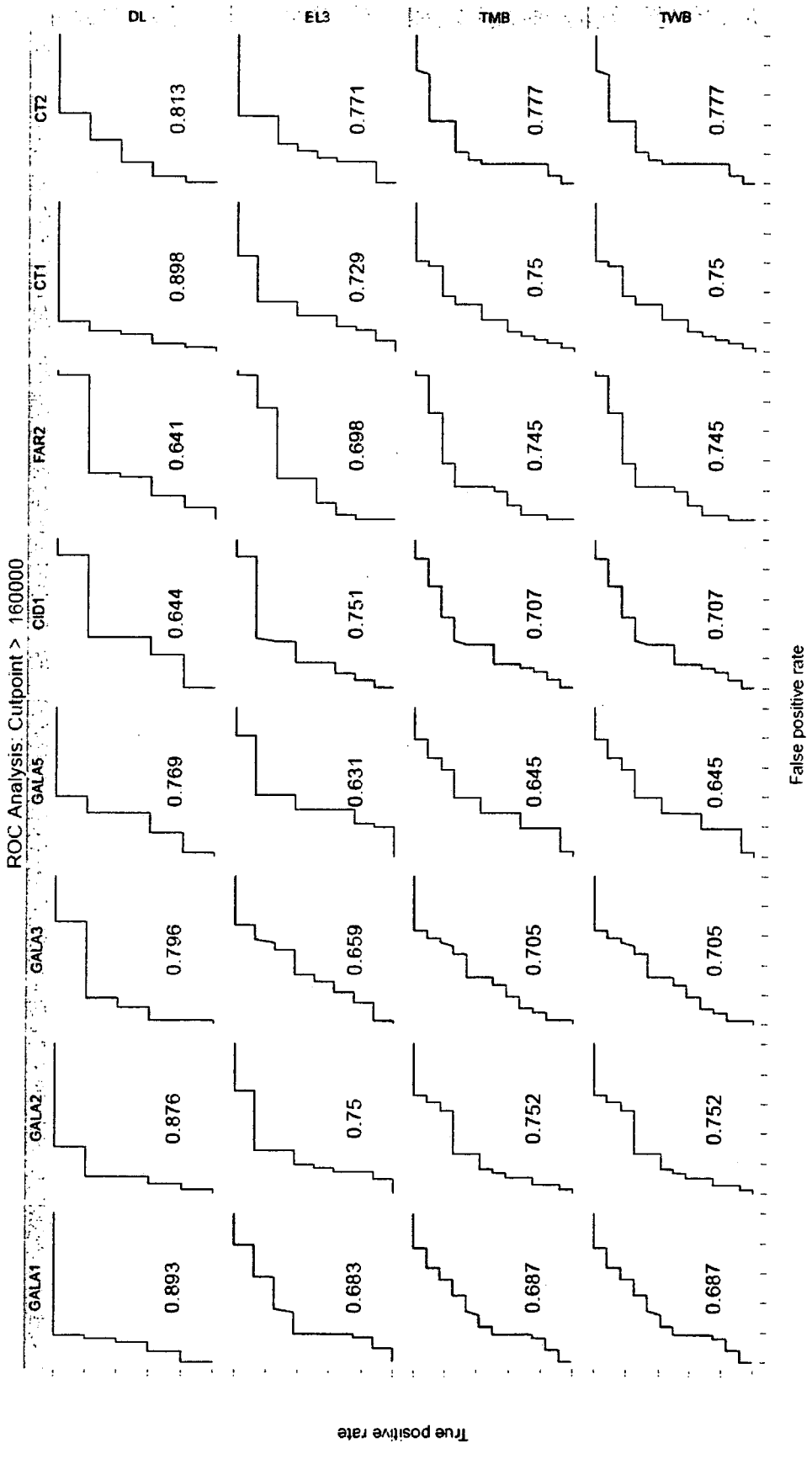
TMB
TWB
CT2
CT1
FAR2
CID1
GALA5
GALA3
GALA2
GALA1
0.822
0.761
0.81
0.777
0.86
0.65
0.723
0.75
0.715
0.691
0.72
0.745
0.693
0.761
0.743
0.707
0.758
0.613
0.682
0.645
0.777
0.619
0.693
0.705
0.848
0.647
0.725
0.752
0.814
0.617
0.675
0.687
True positive rate
False positive rate

Figure 8C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CT1
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GALA5
GALA3
GALA2
GALA1
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0.771
0.777
0.777
0.898
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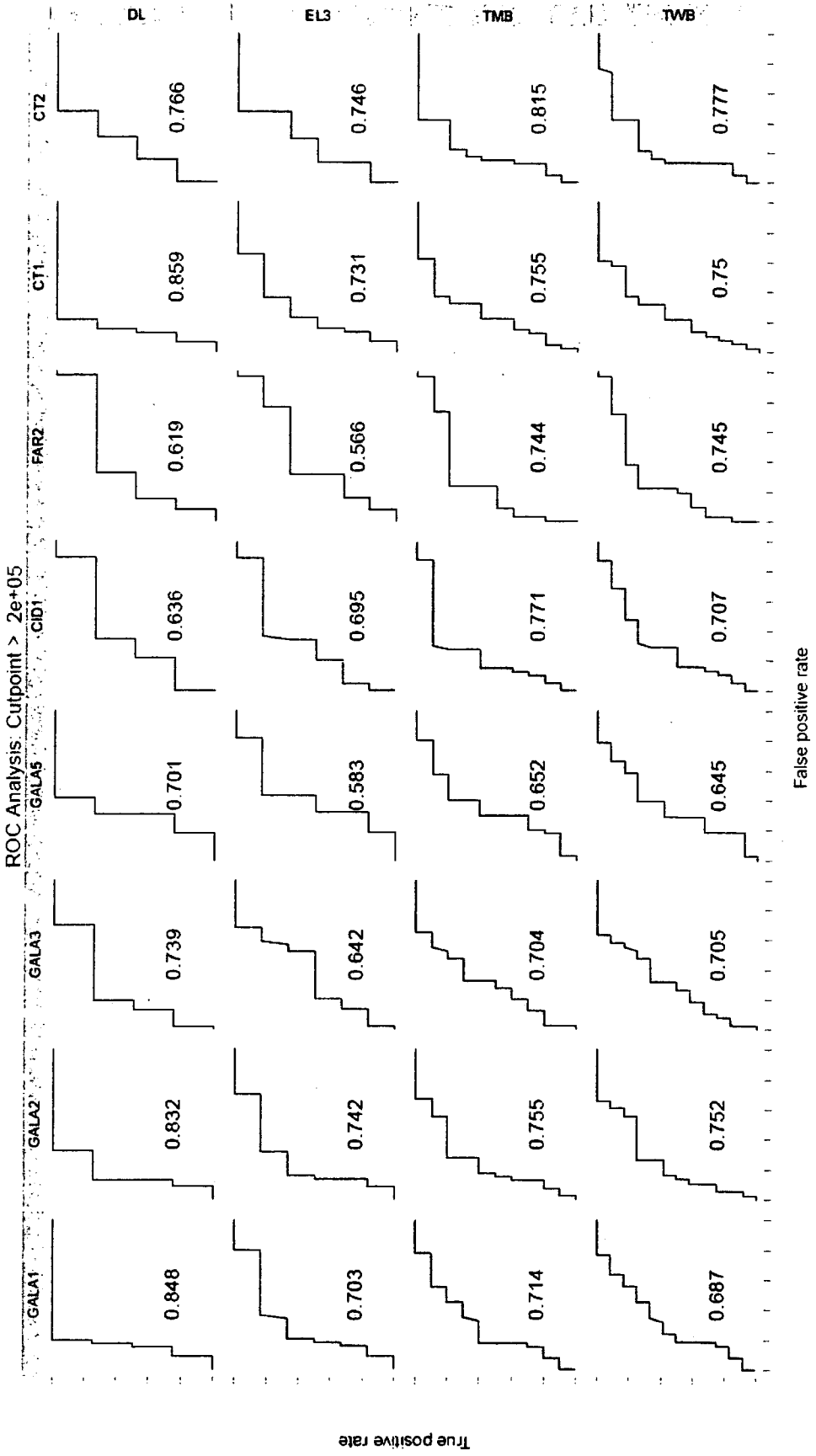
0.75
0.75
0.641
0.698
0.745
0.745
0.644
0.751
0.707
0.707
0.769
0.631
0.645
0.645
0.796
0.659
0.705
0.705
0.876
0.75
0.752
0.752
0.893
0.683
0.687
0.687
True positive rate
False positive rate

Figure 8D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0.745
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0.695
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0.707
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0.645
0.739
0.642
0.704
0.705
0.832
0.742
0.755
0.752
0.848
0.703
0.714
0.687
True positive rate
False positive rate

Figure 8E

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/000616

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOWDALL SAMANTHA M J ET AL: "Characterisation of IgG(T) serum antibody responses to two larval antigen complexes in horses naturally- or experimentally-infected with cyathostomins." INTERNATIONAL JOURNAL FOR PARASITOLOGY, vol. 34, no. 1, January 2004 (2004-01), pages 101-108, XP002594895 ISSN: 0020-7519 cited in the application see section 2.2and p.107, col.2, 3rd paragraph. ----- -/--	1-5,11, 15-19,25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2010	07/09/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hinchliffe, Philippe

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/000616

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOWDALL S M J ET AL: "Antigen-specific IgG(T) responses in natural and experimental cyathostominae infection in horses" VETERINARY PARASITOLOGY, vol. 106, no. 3, 26 June 2002 (2002-06-26), pages 225-242, XP002594896 BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US ISSN: 0304-4017 cited in the application abstract	1-5,11, 15-19,25
X	DOWDALL S M J ET AL: "Purification and analyses of the specificity of two putative diagnostic antigens for larval cyathostomin infection in horses." RESEARCH IN VETERINARY SCIENCE, vol. 75, no. 3, December 2003 (2003-12), pages 223-229, XP002594897 BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US ISSN: 0034-5288 cited in the application abstract	1-5,11, 15-19,25
A	MATTHEWS JACQUELINE B ET AL: "Recent developments in research into the Cyathostominae and Anoplocephala perfoliata" VETERINARY RESEARCH (LES ULIS), vol. 35, no. 4, July 2004 (2004-07), pages 371-381, XP002594898 ISSN: 0928-4249 page 376, column 2	1-25
T	MCWILLIAM HAMISH E G ET AL: "Identification and characterisation of an immunodiagnostic marker for cyathostomin developing stage larvae" INTERNATIONAL JOURNAL FOR PARASITOLOGY, vol. 40, no. 3, March 2010 (2010-03), pages 265-275, XP002594899 ISSN: 0020-7519	1-25

专利名称(译)	马寄生虫检测		
公开(公告)号	EP2414833A1	公开(公告)日	2012-02-08
申请号	EP2010714348	申请日	2010-03-31
申请(专利权)人(译)	MOREDUN研究所		
当前申请(专利权)人(译)	MOREDUN研究所		
[标]发明人	MATTHEWS JACQUI HODGKINSON JANE PROUDMAN CHRISTOPHER		
发明人	MATTHEWS, JACQUI HODGKINSON, JANE PROUDMAN, CHRISTOPHER		
IPC分类号	G01N33/53		
CPC分类号	G01N33/569 G01N33/5308 G01N2333/4353		
优先权	2009005511 2009-03-31 GB		
其他公开文献	EP2414833B1		
外部链接	Espacenet		

摘要(译)

本发明提供了诊断cyathostomin感染的方法，所述方法包括鉴定样品中抗cyathostomin幼虫抗原抗体水平的步骤，其中抗cyathostomin幼虫抗原抗体水平指示cyathostomin感染。