



Universiteit
Leiden
The Netherlands

Circulating gut-associated antigens of *Schistosoma mansoni* : biological, immunological, and molecular aspects

Dam, G.J. van

Citation

Dam, G. J. van. (1995, February 9). *Circulating gut-associated antigens of Schistosoma mansoni : biological, immunological, and molecular aspects*. Retrieved from <https://hdl.handle.net/1887/41317>

Version: Not Applicable (or Unknown)

License:

Downloaded from: <https://hdl.handle.net/1887/41317>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/41317> holds various files of this Leiden University dissertation.

Author: Dam, G.J. van

Title: Circulating gut-associated antigens of Schistosoma mansoni : biological, immunological, and molecular aspects

Issue Date: 1995-02-09

Appendices



I. List of abbreviations

| | |
|----------------------|--|
| 1D | one-dimensional |
| 2D | two-dimensional |
| acq | acquisition |
| AlgG | aggregated human IgG |
| AWA | adult worm antigen |
| AWA-TCA | trichloroacetic acid-soluble fraction of AWA |
| BSA | bovine serum albumin |
| C1q | complement factor 1q |
| CAA | circulating anodic antigen |
| CCA | circulating cathodic antigen |
| CID-MS/MS | collision-induced-dissociation tandem mass spectrometry |
| COSY | scalar-shift correlated spectroscopy |
| DIBA | dot immunobinding assay |
| ELISA | enzyme-linked immunosorbent assay |
| epg | eggs per gram of faeces |
| ESA | excretion and secretion antigens |
| FAB | fast atom bombardment |
| FITC | fluoresceine isothiocyanate |
| Fuc | L-fucose |
| g | gram |
| Gal | D-galactose |
| GAlNAc | N-acetyl-D-galactosamine |
| GARP | globally optimized alternating-phase rectangular pulses |
| GLC | gas-liquid chromatography |
| GlcA | D-glucuronic acid |
| GlcNAc | N-acetyl-D-glucosamine |
| h | hour |
| HMQC | ^1H - ^{13}C -heteronuclear spectroscopy |
| HOHAHA | homonuclear Hartmann-Hahn |
| ID | immunodiffusion |
| IdoA | L-iduronic acid |
| IE | immunoelectrophoresis |
| IFA | immunofluorescence assay |
| kDa | kilodalton |
| Le ^x | Lewis x |
| McAb | monoclonal antibody |
| MEG | major egg glycoprotein |
| min | minutes |
| MS | mass spectrometry |
| MSA _{1,2,3} | major serological antigens 1, 2, and 3 |
| n.O.e. | nuclear Overhauser enhancement |
| Neu5Ac | N-acetylneurameric acid, sialic acid |
| NMR | nuclear magnetic resonance |
| NOESY | nuclear Overhauser enhancement spectroscopy |
| ρ | pyranose |
| PAS | periodic acid-Schiff reaction on carbohydrates |
| PBS | phosphate-buffered saline |
| PT | NaCl/P _t / 0.3% Tween-20 |
| Rha | D-rhamnose |
| SDS-PAGE | sodium dodecylsulfate polyacrylamide gel electrophoresis |
| SEA | soluble egg antigen |
| TCA | trichloroacetic acid |
| TMB | 3,3',5,5'-tetramethylbenzidine, $(\text{Me}_2\text{NH}_2\text{C}_6\text{H}_2^-)_2$ |
| v/v | volume/volume |
| w/v | weight/volume |



II. List of figures and illustrations

| | Page |
|---|------|
| Chapter 1 | |
| Figure 1. Paired adult <i>Schistosoma mansoni</i> worms. | 3 |
| Figure 2. Life cycle of <i>Schistosoma mansoni</i> and <i>Schistosoma haematobium</i> | 4 |
| Figure 3. Portions of male and female schistosome worms showing blood pigments in gut and in regurgitant. | 5 |
| Figure 4. Immunoelectropherogram of an anti-CAA and an anti-CCA McAb against AWA-TCA. | 10 |
| Chapter 2 | |
| Figure 1. Carbohydrate structure found in <i>Schistosoma</i> egg glycoproteins and glycolipids. | 34 |
| Chapter 3 | |
| Figure 1. Immunofluorescence patterns of anti-CAA and anti-CCA McAbs on sections of adult <i>Schistosoma mansoni</i> worms fixed in Rossman's fixative and on frozen sections of livers of <i>Schistosoma mansoni</i> -infected hamsters. | 55 |
| Figure 2. Ultrastructural localization of CAA and CCA on section of syncytium of gut of <i>Schistosoma mansoni</i> | 59 |
| Figure 3. Immunoelectropherograms of anti-CAA McAbs and anti-CCA McAbs against AWA, AWA-TCA, SEA and SEA-TCA. | 61 |
| Figure 4. Immunodiffusion patterns of anti-CAA and anti-CCA McAbs against AWA-TCA and TCA-treated serum and urine of <i>Schistosoma mansoni</i> -infected hamsters. | 62 |
| Figure 5. Dot immunobinding assay patterns of anti-CAA McAbs and anti-CCA McAbs against various antigen preparations. | 63 |
| Figure 6. Immunoblot-patterns of a number of anti-CCA McAbs and controls after electrophoretic separation of AWA on an 8% polyacrylamide gel. | 64 |
| Figure 7. Reactivity of AWA-TCA, immunopurified CAA and CCA preparations in 4 different antigen-capture ELISA's. | 65 |
| Chapter 4 | |
| Figure 1. IFA: recognition of common epitopes on frozen sections of <i>Schistosoma mansoni</i> adult worms in infected hamster liver. | 80 |
| Figure 2. IFA: recognition of common epitopes on sections of <i>Schistosoma mansoni</i> adult male worms fixed in Rossman's fixative. | 81 |
| Figure 3. IFA: recognition of common epitopes on eggs in frozen sections of <i>Schistosoma mansoni</i> -infected hamster liver. | 84 |
| Figure 4. Inhibition DIBA of McAbs 114-3A12-A and 114-3C8-A with infected and normal human serum. | 85 |
| Figure 5. Immunoelectrophoresis of 0.8 mg AWA and 0.4 mg AWA-TCA against McAb 54-1F6-A (ascitic fluid). | 86 |
| Chapter 5 | |
| Figure 1. Semi-thin section of LR White-embedded adult <i>Schistosoma mansoni</i> labeled with anti-CCA McAb-FITC. | 97 |
| Figure 2. Localization of CCA in adult <i>Schistosoma mansoni</i> using anti-CCA McAb-FITC followed by anti-FITC-gold. | 97 |
| Figure 3. Section through the gut of adult <i>Schistosoma mansoni</i> incubated with pooled sera from infected humans. | 97 |
| Figure 4. Section through the tegument of adult <i>Schistosoma mansoni</i> incubated with pooled sera from infected humans. | 97 |

**Chapter 6**

| | | |
|-----------|---|-----|
| Figure 1. | Titration of coating antibody 54–5C10–A and CCA in the indirect and direct ELISA. | 108 |
| Figure 2. | Influence of post-coating with 0.5% BSA in PBS. | 109 |
| Figure 3. | Serum dilution curves of 3 individual sera, including the positive control serum, in the indirect, direct, and Ab-capture ELISA. | 110 |
| Figure 4. | Correlation scattergrams of sample/reference ratios of human IgM anti-CCA antibodies obtained by three variant ELISA procedures and by the IFA. | 112 |

Chapter 7

| | | |
|-----------|---|-----|
| Figure 1. | Detection of CCA before and after reductive β -elimination. | 128 |
| Figure 2. | Elution pattern of reductive alkaline treated <i>Schistosoma mansoni</i> CCA on a column of Bio-Gel P-6. | 129 |
| Figure 3. | Partial FAB mass spectrum of permethylated fraction O14, CID mass spectrum and fragmentation scheme for <i>m/z</i> 961 from permethylated fraction O14, and CID mass spectrum and fragmentation scheme for <i>m/z</i> 1165 from permethylated fraction O14. | 132 |
| Figure 4. | Resolution-enhanced 500-MHz ^1H -NMR spectrum of fraction P. | 136 |
| Figure 5. | 500-MHz 2D NOESY spectrum of fraction P. | 138 |
| Figure 6. | Binding of anti-i and anti-l antibodies to CCA in ELISA. | 140 |
| Figure 7. | Influence of mild acid hydrolysis of CCA on reactivity in ELISA. | 140 |
| Figure 8. | Inhibition of McAb binding to CCA in antigen-coated ELISA. | 141 |
| Figure 9. | Recognition of CCA in antigen-coated ELISA by different McAbs and lectins. | 142 |

Chapter 8

| | | |
|-----------|--|-----|
| Figure 1. | Detection of CAA-specific immunogenicity in AWA-TCA before and after alkaline borohydride treatment, using both the antigen-capture ELISA and the direct antigen-coated ELISA. | 159 |
| Figure 2. | Detection of CAA and CCA in neutral and negatively charged Mono Q fractions of native and alkaline borohydride-treated immunopurified CAA. | 160 |
| Figure 3. | Resolution-enhanced 500-MHz 1D ^1H NMR spectrum of fraction CAA-P. | 162 |
| Figure 4. | 75-MHz 1D noise ^1H -decoupled ^{13}C NMR spectrum of fraction CAA-P. | 164 |
| Figure 5. | 600-MHz 2D HMQC spectrum of fraction CAA-P. | 165 |
| Figure 6. | 500-MHz 2D NOESY spectrum of fraction CAA-P. | 166 |

Chapter 9

| | | |
|-----------|---|-----|
| Figure 1. | Binding of McAbs to human granulocytes as measured by flow cytometry. | 179 |
| Figure 2. | Relative anti-CCA reactivity of IgM and IgG antibodies as determined by ELISA in sera of different groups of patients. | 180 |
| Figure 3. | Lysis of granulocytes by sera of different groups of patients. | 181 |
| Figure 4. | Correlation of granulocyte lysis by human sera with anti-CCA reactivity as measured in ELISA for anti-CCA IgM or anti-CCA IgG antibodies. | 182 |

Chapter 10

| | | |
|-----------|---|-----|
| Figure 1. | Binding of <i>Schistosoma mansoni</i> CAA to complement components in ELISA. | 196 |
| Figure 2. | Inhibition by soluble C1q of the binding of CAA to C1q-coated plates. | 196 |
| Figure 3. | Inhibition by soluble collagen type 1 of the binding of CAA to C1q-coated plates. | 197 |
| Figure 4. | Binding of <i>Schistosoma mansoni</i> CAA and CCA to C1q fragments in ELISA. | 198 |
| Figure 5. | Binding of <i>Schistosoma mansoni</i> CAA and CCA to C1q. | 199 |
| Figure 6. | Activation of precursor C1 by <i>Schistosoma mansoni</i> antigen preparations. | 200 |

**Chapter 11**

| | | |
|-----------|--|-----|
| Figure 1. | Excretion of CAA and CCA by in vitro developing schistosomula. | 211 |
| Figure 2. | Excretion of CAA and CCA per schistosomulum after 11 days of culture: difference after feeding erythrocytes at day 7. | 211 |
| Figure 3. | Excretion of CAA and CCA by schistosomula incubated without or with 5×10^{-4} M colchicine in the culture medium. | 212 |
| Figure 4. | Change in ratio of CAA and CCA in culture supernatants of schistosomula fed at day 7 and not fed at any time. | 213 |
| Figure 5. | Detection of CAA and CCA in sera of Swiss mice during the development of an infection with 1000 <i>Schistosoma mansoni</i> cercariae. | 214 |
| Figure 6. | Detection and association of CAA and CCA in serum and urine samples of individual mice at day 45 after infection with 1000 <i>Schistosoma mansoni</i> cercariae. | 215 |
| Figure 7. | <i>In vitro</i> excretion of circulating antigens by 7 week-old adult worms removed from infected hamsters. | 216 |



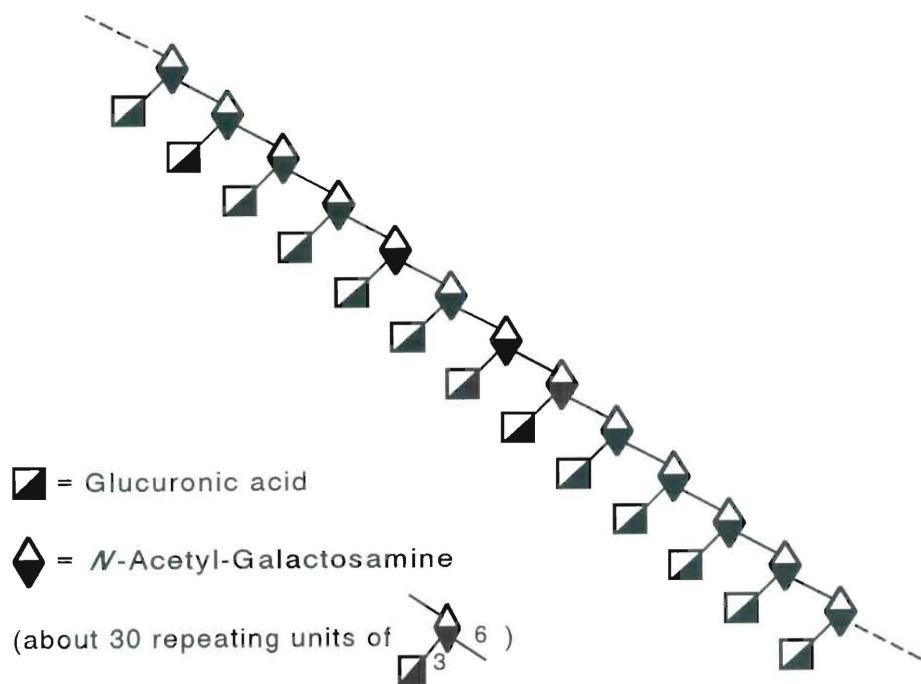
III. List of tables

| | Page |
|--|------|
| Chapter 1 | |
| Table 1. Characteristics of circulating anodic antigen. | 8 |
| Table 2. Characteristics of circulating cathodic antigen. | 9 |
| Chapter 2 | |
| Table 1. Examples of oligomannose, <i>N</i> -acetyllactosamine, hybrid and xylose-containing types of asparagine-linked carbohydrate chains. | 22 |
| Table 2. Core structures found in mucin-type serine- or threonine-linked oligosaccharides and polysaccharides. | 23 |
| Table 3. Various lectins used for the analysis of carbohydrates in schistosomal antigens. . | 25 |
| Table 4. Lectin binding to <i>Schistosoma</i> adult worms and schistosomula. | 27 |
| Table 5. Schistosome carbohydrate structures described by Cummings and co-workers. . | 29 |
| Table 6. Lectin binding to <i>Schistosoma</i> eggs. | 33 |
| Table 7. Lectin binding to <i>Schistosoma cercariae</i> and newly transformed schistosomula ($\leq 3h$). | 37 |
| Chapter 3 | |
| Table 1. Infection and immunization protocols for the various fusion numbers of the hybridomas used in this study. | 52 |
| Table 2. Monoclonal antibodies recognizing CAA. | 56 |
| Table 3A. Monoclonal antibodies recognizing CCA. | 57 |
| Table 3B. McAbs combined under one representative McAb in Table 3A. | 58 |
| Table 4. Immunofluorescence reaction of anti-CAA and anti-CCA monoclonal antibodies on different life cycle stages of <i>Schistosoma mansoni</i> | 58 |
| Chapter 4 | |
| Table 1. IFA: numbers of McAbs recognizing common epitopes present on various tissues of cryostat sections of livers of <i>Schistosoma mansoni</i> -infected hamsters. . | 79 |
| Table 2. IFA: numbers of McAbs recognizing common epitopes present on various tissues of paraffin sections of <i>Schistosoma mansoni</i> male worms fixed with Rossman's fixative. | 82 |
| Table 3. DIBA: numbers of McAbs recognizing various <i>Schistosoma mansoni</i> antigen preparations. | 82 |
| Chapter 6 | |
| Table 1. Values of average S/R ratio's and standard deviations for 50 negative control sera in indirect, direct and Ab-capture ELISA. | 111 |
| Table 2. Intra-assay and inter-assay variability of S/R ratio's for a serum of moderate activity in indirect, direct and Ab-capture ELISA. | 111 |
| Table 3. Pearson product-moment correlation coefficient r for the association between the three ELISA's and the IFA. | 111 |
| Chapter 7 | |
| Table 1. Summary of ions observed, with their assignments, on FAB-MS and CID-MS/MS analysis of permethylated carbohydrate-containing fractions isolated after reductive β -elimination of CCA. | 126 |
| Table 2. 1H -Chemical shifts of structural-reporter-group protons of the constituent monosaccharides for oligosaccharide alditols derived from <i>Schistosoma mansoni</i> CCA. | 130 |

| | | |
|-------------------|---|-----|
| Table 3. | ¹ H-Chemical shifts of structural-reporter-group protons of the constituent monosaccharides for the polysaccharide alditol fraction P, derived from <i>Schistosoma mansoni</i> CCA. | 135 |
| Table 4. | Sensitivity to periodate oxidation of epitopes recognized by six different CCA-specific McAbs. | 141 |
| Chapter 8 | | |
| Table 1. | ¹ H NMR chemical shifts of the protons of the constituent monosaccharides of the polysaccharide alditols (CAA-P [*]), having repeating →6)-[β-GlcP-(1→3)]-β-GalpNAc-(1→ units, derived from <i>Schistosoma mansoni</i> circulating anodic antigen. | 161 |
| Table 2. | ¹³ C NMR chemical shifts of the carbons of the constituent monosaccharides of the polysaccharide alditols (CAA-P [*]), having repeating →6)-[β-GlcP-(1→3)]-β-GalpNAc-(1→ units, derived from <i>Schistosoma mansoni</i> circulating anodic antigen, together with those of reference β-GlcP and β-GalpNAc-(1→6)-β-GalpNAc. | 163 |
| Chapter 9 | | |
| Table 1. | Reactivity of native sera and immunopurified human anti-CCA antibodies in ELISA and granulocytotoxicity assay. | 182 |
| Chapter 10 | | |
| Table 1. | Binding of C1 and C1q to <i>Schistosoma mansoni</i> AWA-TCA, CAA and controls BSA and AlgG, determined in a C1q hemolytic assay. | 197 |
| Chapter 11 | | |
| Table 1. | Correlations between antigen concentrations and number of worms in serum and urine of mice 45 days after an infection with 1000 <i>Schistosoma mansoni</i> cercariae. | 214 |
| Table 2. | Detectability of circulating antigens in serum of animals experimentally infected with different <i>Schistosoma</i> species. | 218 |

**IV. Carbohydrate structures found in CAA**

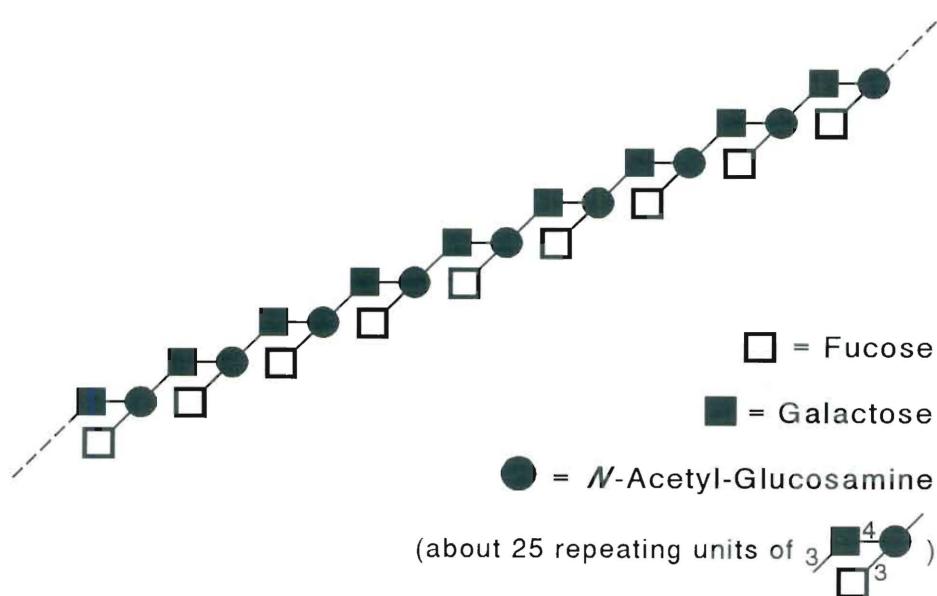
polysaccharide



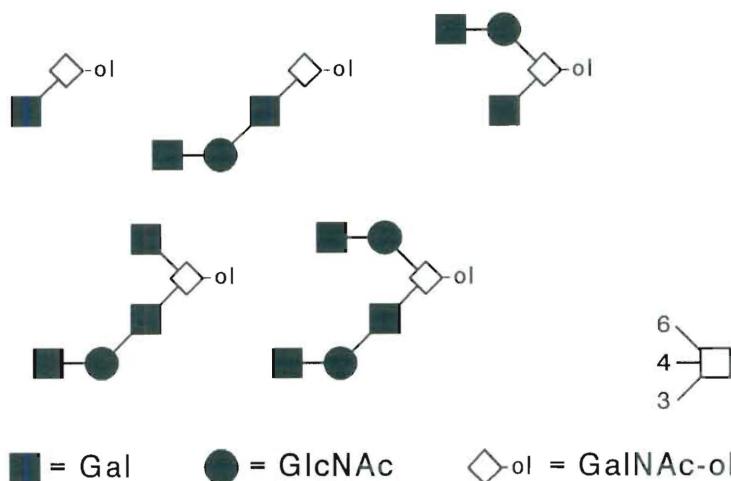


V. Carbohydrate structures found in CCA

polysaccharide



oligosaccharides







General acknowledgements

This thesis was realized thanks to the invaluable help of the co-authors, my wife, and many other people inside and outside the Laboratory for Parasitology in Leiden.

I appreciated in particular the discussions with and help of the following people: Aldert Bergwerff, André Deelder, Yvonne Fillié, Niels de Jonge, Dieuwke Cornelis, Lisette van Lieshout, Peter Rotmans, and Maria Yazdanbakhsh.

This study received financial support from a grant (No. 881-429-021) from the Netherlands Foundation for Biological Research (NWO/BION).





Curriculum vitae

Govert van Dam werd geboren op 12 augustus 1960 te Gouda. Van 1966 tot 1972 doorliep hij de lagere school aan de School met de Bijbel in Langerak. In 1978 behaalde hij het Gymnasium B diploma aan het Christelijk Lyceum-HAVO in Gouda. In datzelfde jaar begon hij met de studie scheikunde aan de Rijksuniversiteit Utrecht, waarvan hij het volgende jaar de propaedeuse haalde, in 1983 het kandidaatsexamen aflegde (cum laude) en in 1987 het doctoraalexamen. In de doctoraalfase werden de keuzevakken Biofysica (prof. Y.K. Levine), Analytische Chemie (tevens afstudeerrichting, prof. C.L. De Ligny), en Immunologie (prof. J.M.N. Willers) gedaan alsook de onderwijsbevoegdheden voor Natuurkunde en Scheikunde verkregen. Tijdens deze periode deed hij van 1979 tot 1980 een propaedeutisch jaar ter voorbereiding op universitaire studie aan de Evangelische Hogeschool te Amersfoort en verrichtte hij van 1985 tot 1986 een stage Immunologie aan Karelsuniversiteit te Praag, Tsjechië (Dr. J. Kocourek, Dr. K. Bezouška). Van 1988 tot 1992 was Govert van Dam verbonden aan het Laboratorium voor Parasitologie in Leiden als Onderzoeker in Opleiding op BION–projekt "Onderzoek naar darm–gebonden antigenen van *Schistosoma mansoni*: Immunochemische analyse en bestudering van de immunologische respons van de gastheer", onder begeleiding van Prof. Dr. A.M. Deelder en Dr. J.P. Rotmans. Binnen hetzelfde laboratorium werd hij op 1 maart 1993 voor twee jaar als onderzoeker aangesteld in het kader van het STD3 (EG)–projekt "Epidemiology, serology and chemotherapy of *Schistosoma* in a recently exposed community near Richard Toll, Senegal".

En dan nog iets, een laatste waarschuwing. Er worden veel te veel boeken geschreven en van al dat lezen word je alleen maar moe. Na alles wat hier gezegd is, valt er alleen nog dit te zeggen: heb ontzag voor God en onderhoud zijn geboden; daar komt het voor de mens op aan. Want God zal oordelen over alles wat je doet, zelfs over wat verborgen blijft, of het nu goed is of slecht.

(Prediker 12 vers 12 – 14)

My son, there is something else to watch out for. There is no end to the writing of books, and too much study will wear you out. After all this, there is only one thing to say: Fear God, and obey his commands, because this is all that man was created for. God is going to judge everything we do, whether good or bad, even things done in secret.

(*Ecclesiastes* 12 verse 12 – 14)

Soli Deo Gloria

