Comparison Crude Antigens of Fasciolosis hepatica and Candida albicans by Molecular Tests: A way to Drug

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ABSTRACT

Early diagnosis is very important for fasciolosis and Candidiasis in prevention and control. Two productive species, namely F. hepatica and Candida Albicans of the disease in terms of together morphological similarities in some of the features of a great but the differences. In this study, the presence or absence of similarity between genes raw of Antique excretory - secretory and somatic two species of the parasite and Fungi to Elisa test was evaluated. Crude antigens of both species (including excretory - secretory and SCC) developed and to use in the cold ºc -20 were kept. In the preparing the antiserum, antigens with the increasing the dose 0 / 5-2 / 5 in the five times with intervals of seven days, two rabbits were injected. Ten days after the last injection, blood and serum samples after separation, in cold 20 ° C were kept. Reaction of Crude antigen the antiserum any species with the self antiserum (homologous) and as well as antiserum other species (heterologous) evaluated by ELISA and ELISA values were recorded. When the reaction with the antiserum-antigen ELISA values of any other species in comparison with control samples showed that with the antiserum antigen any kind other species show that a strong reaction. Meaning that of antigenic cross-reactivity in ELISA two species is similar and there. However, antigens of F. hepatica and Candida Albicans with the antiserum a stronger reaction than the antiserum were found Despite the similarity between many of antigenic material of two species, some of these substances are different. F. hepatica and Candida Albicans antigenic differences between the materials is not enough to prevent cross-reaction between the two species and more research is geared in ELISA for the detection, isolation and purification of antigenic materials of any recommended.

Keywords: Molecular Tests, Fasciolosis Hepatica, Candida Albicans.

INTRODUCTION

Fasciola hepatica, also known as the common liver fluke or sheep liver fluke, is a parasitic trematode (fluke or flatworm, a type of helminth) of the class Trematoda, phylum Platyhelminthes. It infects the livers of various mammals, including humans. The disease caused by the fluke is called fascioliasis or fasciolosis, which is a type of helminthiasis and has been classified as a neglected tropical disease [1]. Fascioliasis is currently classified as a plant/food-borne trematode infection, often acquired through eating the parasite metacercariae encysted on plants [2]. F. hepatica, which is distributed worldwide has been known as an important parasite of sheep and cattle for many years and causes great economic losses to these livestock species. Because of its size and economic importance, it has been the subject of many scientific investigations and may be the best-known of any trematode
species. F. Hepatica's closest relative is Fasciola gigantica. These two flukes are sister species, they share many morphological features and can mate with each other [3]. Fasciola hepatica is one of the largest flukes of the world, reaching a length of 30 mm and a width of 13 mm (Fasciola gigantica, on the other hand, is even bigger and can reach up to 75 mm). It is leaf-shaped, pointed at the back (posteriorly) and wide in the front (anteriorly). The oral sucker is small but powerful and is located at the end of a cone-shape projecting at the anterior end. The acetabulum is a larger sucker than the oral sucker and is located at the anterior end [4].

Currently, F. hepatica has the widest geographical spread of any parasitic and vector-borne disease. Originating in Europe, it has expanded to colonise over 50 countries, covering all continents except Antarctica [5]. In contrast, F. gigantica is generally considered more geographically constricted to the tropical regions of Africa, Asia and the Middle East, there is some overlap between the two species [6].

Climate and Seasonal Influence - Climate affects both F. hepatica itself and its definitive host, the snail [7]. For example, the development of F. hepatica miracidia and larvae, and the reproduction of Galba truncatula, require a temperature range of 10-25 °C. In addition to this, they both require high levels of moisture in the air, as both are at risk of desiccation [8]. Due to this, the prevalence, along with the intensity of infection, of F. hepatica is primarily dependent on rainfall levels and temperature [9].

For more information on the epidemiology – see the disease page, fascioliasis Infection begins when cyst-covered aquatic vegetation is eaten or when water containing metacercariae is drunk. In the United Kingdom, F. hepatica frequently causes disease in ruminants, most commonly between March and December [10]. Humans become infected by eating watercress or by drinking 'Emoliente', a Peruvian drink that uses drops of watercress juice. Cattle and sheep are infected when they consume the infectious stage of the parasite from low-lying, marshy pasture [10]. Human infections occur in parts of Europe such as England and Ireland; also in Cuba and South America especially in the Altiplano regions of the Peruvian and Bolivian Andes. Infections are emerging now in Vietnam and Cambodia. As of 2014, in the cattle farming areas near Cusco, Peru, the prevalence of infection in children between 3 and 12 years was 11% by stool microscopy and ELISA. Risk factors were number of siblings in the household, drinking untreated water and giardiasis [11]. Fascioliasis is an important cause of both production and economic losses in the dairy and meat industry. Over the years, the prevalence has increased and it is likely to continue increasing in the future [12]. Livestock are often treated with Flukicides, these are chemicals that are toxic to flukes. The two chemicals used are triclabendazole and bithionol. Ivermectin, which is widely used for many helminthic parasites, has low effectiveness against F. hepatica, as does praziquantel [13]. For humans, the type of control depends on the setting. One important method is through the strict control over the growth and sales of edible water plants such as watercress. This is particularly important in highly endemic areas. Some farms are irrigated with polluted water, hence, vegetables farmed from such land should be thoroughly washed and cooked before being eaten [14].

The presence of F. hepatica can interfere with the detection of bovine tuberculosis in cattle. Cattle co-infected with F. hepatica, compared to those infected with M. bovis alone, react weakly to the single intradermal comparative cervical tuberculin (SICCT) test. Therefore, an infection from F. hepatica can make it difficult to detect bovine tuberculosis, this is, of course, a major problem in the farming industry [15].

For more information on the diagnosis – see the disease page, fascioliasis A diagnosis may be made by finding yellow-brown eggs in the stool. They are indistinguishable from the eggs of Fascioloides magna, although the eggs of F. magna are very rarely passed in sheep, goats, or cattle. If a patient has eaten infected liver, and the eggs pass through the body and out via the faeces, a false positive result to the test can occur. Daily examination during a liver-free diet will unmask this false diagnosis [16].

An enzyme-linked immunosorbent assay (ELISA) test is the diagnostic test of choice. ELISA is available commercially and can detect anti-hepatica antibodies in serum and milk; new tests intended for use on faecal samples are being developed [17]. Using ELISA is more specific than using a Western blot or Arc2 immunodiffusion [18]. Proteases secreted by F. hepatica have been used experimentally in immunizing antigens. Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans [19], and candidal onychomycosis, an infection of the nail plate. Systemic fungal infections (fungemias) including those by C. albicans have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). Candida albicans biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by C. albicans have become a cause of major health concerns. About 85-95% of vaginal infections cases are responsible for physician office visits every year [20].
C. albicans is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. Overgrowth of the fungus results in candidiasis (candidosis). Candidiasis is often observed in immunocompromised individuals, including HIV-infected patients [21]. A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, which is usually easily cured in people who are not immunocompromised. For example, higher prevalence of colonization of C. albicans was reported in young individuals with tongue piercing, in comparison to unpierced matched individuals [22]. To infect host tissue, the usual unicellular yeast-like form of C. albicans reacts to environmental cues and switches into an invasive, multicellular filamentous form, a phenomenon called dimorphism [23]. In addition, an overgrowth infection is considered superinfection, usually applied when an infection become opportunistic and very resistant to antifungals. It then becomes suppressed by antibiotics. The infection is prolonged when the original sensitive strain is replaced by the antibiotic-resistant strain.

One of the most important features of the C. albicans genome is the occurrence of numeric and structural chromosomal rearrangements as means of generating genetic diversity, named chromosome length polymorphisms (contraction/expansion of repeats), reciprocal translocations, chromosome deletions and trisomy of individual chromosomes. These karyotypic alterations lead to changes in the phenotype, which is an adaptation strategy of this fungus [24]. These mechanisms will be better understood with the complete analysis of the C. albicans genome.

An unusual feature of the Candida genus is that in many of its species (including C. albicans and C. tropicalis, but not, for instance, C. glabrata) the CUG codon, which normally specifies leucine, specifies serine in these species. This is an unusual example of a departure from the standard genetic code, and most such departures are in start codons or, for eukaryotes, mitochondrial genetic codes [16]. This alteration may, in some environments, help these Candida species by inducing a permanent stress response, a more generalized form of the heat shock response [20]. The genome of C. albicans is highly dynamic, and this variability has been used advantageously for molecular epidemiological studies and population studies in this species. The genome sequence has allowed for identifying the presence of a parasexual cycle (no detected meiotic division) in C. albicans [25]. This study of the evolution of sexual reproduction in six Candida species found recent losses in components of the major meiotic crossover-formation pathway, but retention of a minor pathway [26]. The authors suggested that if Candida species undergo meiosis it is with reduced machinery, or different machinery, and indicated that unrecognized meiotic cycles may exist in many species. In another evolutionary study, introduction of partial CUG identity redefinition (from Candida species) into Saccharomyces cerevisiae clones caused a stress response that negatively affected sexual reproduction. This CUG identity redefinition, occurring in ancestors of Candida species, was thought to lock these species into a diploid or polyploid state with possible blockage of sexual reproduction.

Although often referred to as "dimorphic", C. albicans is in fact polyphenic. When cultured in standard yeast laboratory medium, C. albicans grows as ovoid “yeast” cells. However, mild environmental changes in temperature and pH can result in a morphological shift to pseudohyphal growth [23]. Pseudohyphae share many similarities with yeast cells [22], but their role during candidiasis remains unknown. When C. albicans cells are grown in a medium that mimics the physiological environment of a human host, they grow as “true” hyphae. Its ability to form hyphae has been proposed as a virulence factor, as these structures are often observed invading tissue, and strains that are unable to form hyphae are defective in causing infection. Candida albicans can also form Chlamydospores, the function of which remains unknown [27]. Round, white-phase and elongated, opaque-phase Candida albicans cells: the scale bar is 5 μm. In this model of the genetic network regulating the white-opaque switch, the white and gold boxes represent genes enriched in the white and opaque states, respectively. The blue lines represent relationships based on genetic epistasis. Red lines represent Wor1 control of each gene, based on Wor1 enrichment in chromatin immunoprecipitation experiments. Activation (arrowhead) and repression (bar) are inferred based on white- and opaque-state expression of each gene.

In a process that superficially resembles dimorphism, C. albicans undergoes a process called phenotypic switching, in which different cellular morphologies are generated spontaneously. Of the classically studied strains, one that undergoes phenotypic switching is WO-1 [28], which consists of two phases: one that grows as round cells in smooth, white colonies and one that is rod-like and grows as flat, gray colonies. The other strain known to undergo switching is 3153A; this strain produces at least seven different colony morphologies. In both the WO-1 and 3153A strains, the different phases convert spontaneously to the other(s) at a low frequency. The switching is reversible, and colony type can be inherited from one generation to another. While several genes that are expressed differently in different colony morphologies have been identified, some recent efforts focus on what might control these changes. Further, whether a potential molecular link between dimorphism and phenotypic switching occurs is a tantalizing question [29].
In the 3153A strain, a gene called SIR2 (for silent information regulator), which seems to be important for phenotypic switching, has been found. SIR2 was originally found in Saccharomyces cerevisiae (brewer's yeast), where it is involved in chromosomal silencing—a form of transcriptional regulation, in which regions of the genome are reversibly inactivated by changes in chromatin structure (chromatin is the complex of DNA and proteins that make chromosomes) [30]. In yeast, genes involved in the control of mating type are found in these silent regions, and SIR2 represses their expression by maintaining a silent-competent chromatin structure in this region [31]. The discovery of a C. albicans SIR2 implicated in phenotypic switching suggests it, too, has silent regions controlled by SIR2, in which the phenotype-specific genes may reside.

Another potential regulatory molecule is Efg1p, a transcription factor found in the WO-1 strain that regulates dimorphism, and more recently has been suggested to help regulate phenotypic switching. Efg1p is expressed only in the white and not in the gray cell-type, and overexpression of Efg1p in the gray form causes a rapid conversion to the white form [32].

So far, very few data suggest dimorphism and phenotypic switching use common molecular components. However, it is not inconceivable that phenotypic switching may occur in response to some change in the environment, as well as being a spontaneous event. How SIR2 itself is regulated in S. cerevisiae may yet provide clues as to the switching mechanisms of C. albicans.

During both superficial and systemic infection, C. albicans and Fasciolosis Hepatica relies on a battery of virulence factors and fitness attributes. The major factors and fitness traits are discussed below.

**MATERIALS AND METHODS**

This study was descriptive experimental. After production of antiserum against Crude antigens both species of parasites in rabbits In vitro, the reaction between crude antigens produced any species with antiserum produced against other species in ELISA test have been studied. Adult species of F. hepatica and C. albicans from bile ducts of slaughtered at the slaughterhouse sheep inside the physiologic serum were collected and transferred to Pathology laboratory. For the preparation antigens excreted - secreted live species after they were washed several times in Phosphate Buffer Saline (PBS) solution, for six hours at a temperature of 37 degrees was in PBS dissolved, The obtained liquid containing fecal matter – secretary the species, was centrifuged for 35 minutes, The supernatant was stored until use in cold 20 °C [33]. For the preparation somatic antigens fresh species after washing, first thoroughly oscillator in a porcelain mortar and in PBS solution for was homogenized. Then were sonication for 15 minutes in cold conditions. The obtained solution was centrifuged for 35 minutes, and the supernatant was stored until use at -20 °C cold. For the preparation anti-sera from 15 healthy white lab rabbits to weighing 2 kg were used. First rabbits were divided to five groups of three. Groups of one to four respectively to prepare antisera against Excretory-secretory and somatic antigens of F. hepatica and C. albicans were used and the group five were considered as the control group. Antigens of intended protein concentrations of two milligrams per milliliter and were injected subcutaneously. Antigens with a dose of 0.5 to 2.5 ml in five innings and seven day rabbits were injected. The first in injection of complete Freund's adjuvant along with Antigens, a second injection of complete Freund's adjuvant without Antigens was used at the same volume by volume. Ten days after the last injection blood samples of rabbit heart surgery was performed, after separating serum in sterile conditions, the heads were stored until use in the cold. Group of five simultaneous rabbit serum also were prepared to be used as negative controls in experiments.

**RESULTS**

Reaction between excretory-secretory and somatic Antigens of F. hepatica and C. albicans with homologous and heterologous antiserum them were tested in ELISA experiment. Amounts of OD in the Reaction between Antigens and antiserum are provided in Tables 1 and 2.

**Table 1. Amounts OD in the reaction between antigens excreted - secreted homologous and heterologous strains of the Fasiola and Candida antiserum with them**

<table>
<thead>
<tr>
<th>Antigens</th>
<th>C. albicans antiserum</th>
<th>F. Hepatica antiserum</th>
<th>Control Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>0.69</td>
<td>0.60</td>
<td>0.02</td>
</tr>
<tr>
<td>F. Hepatica</td>
<td>0.24</td>
<td>0.52</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Candidiasis is a fungal infection caused by yeasts that belong to the genus Candida. There are over 20 species of Candida yeasts that can cause infection in humans, the most common of which is Candida albicans [34]. Candida yeasts normally live on the skin and mucous membranes without causing infection; however, overgrowth of these organisms can cause symptoms to develop. Symptoms of candidiasis vary depending on the area of the body that is infected [35].

Candidiasis that develops in the mouth or throat is called “thrush” or oropharyngeal candidiasis. Candidiasis in the vagina is commonly referred to as a “yeast infection.” Invasive candidiasis occurs when Candida species enter the bloodstream and spread throughout the body. Click the links below for more information on the different types of Candida infections.
Candida albicans is an opportunistic fungal pathogen that is responsible for candidiasis in human hosts. C. albicans grow in several different morphological forms, ranging from unicellular budding yeast to true hyphae with parallel-side wall [36]. Typically, C. albicanslve as harmless commensals in the gastrointestinal and genitourinary tract and are found in over 70% of the population. Overgrowth of these organisms, however, will lead to disease, and it usually occurs in immunocompromised individuals, such as HIV-infected victims, transplant recipients, chemotherapy patients, and low birth-weight babies [37]. There are three major forms of disease: oropharyngeal candidiasis, vulvovaginal candidiasis, and invasive candidiasis. Over 75% of women will suffer from a C. albicans infection, usually vulvovaginal candidiasis, in their lifetimes, and 40-50% of them will have additional occurrences(s). Interestingly, C. albicans are the 4th leading cause for nosocomial infections in patients’ bloodstream. This could result in an extremely life-threatening, systemic infection in hospital patients with a mortality rate of 30% [38]. For oropharyngeal candidiasis, infection occurs in the mouth or throat, and is identified by white plaque growth on oral mucous membranes. Vulvovaginal candidiasis or a “yeast infection” is the overgrowth of C. albicans in the vagina, and results in rash, itchiness, and discharge from the genital region. Lastly, invasive candidiasis occurs when the fungal pathogen enters the bloodstream and can easily spread to organs throughout the body. Invasive candidiasis is best identified when antibiotics fail to cure a patient’s fever [39]. C. albicans infections are usually treatable with fluconazole, while severe infections require amphotericin B.

Candida albicans is a polymorphic fungus that can grow in several different forms, primarily yeast, pseudohyphae, and hyphae. For its pathogenicity, its ovoid-shaped budding yeast and parallel-walled true hyphae forms are the most important. The hyphae form is more prevalent for an infection, while the yeast form is believed to be important in the spread of C. albicans. The role of pseudohyphae is not very well understood, other than being an intermediate form between yeast and hyphae [40]. Several factors can cause a change in morphology, such as pH differences, temperature changes, carbon dioxide levels, starvation, and quorum-sensing molecules (farnesol, tyrosol, and dodecanol) [41].

Candida albicans have special sets of glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins that allow it to adhere to the surfaces of microorganisms. These glycoproteins are encoded by 8 sets of agglutinin-like sequence (ALS) genes, ranging from Als1-7 and Als9. For adhesion, the Als3 gene appears to be the most important as it is upregulated during an infection of oral and vaginal epithelial cells. Also, it helps with biofilm formation by helping with adhesion to each other [42].

Candida albicans secretes main classes of hydrolases: proteases, phospholipases and lipases. It is proposed that these hydrolases help facilitate the pathogen’s active penetration into host cells and the uptake of extracellular nutrients from the environment. There are about 10 known secreted aspartic proteases (Sap1-10), and their exact contribution to pathogenicity is controversial. For phospholipases, there are 4 major classes (A, B, C, and D), and all 5 members of the B class are involved with the disruption of a host cell surface. Thirdly, lipases are consisted of 10 members (LIP1-10), and studies show that there is decreased virulence in their absent [42].

Fasciolagigantica very rarely infects humans. Reported cases are mainly from Africa. The life cycle, transmission, morphology, clinical presentation, and treatment of the F. gigantica trematode and its infections are very similar to those of F. hepatica.

The Adult Worm - Averaging 30mm in length and 13 mm in width, Fasciola hepatica is one of the largest flukes in the world. The adult worm has a very characteristic leaf shape with the anterior end being broader than the posterior end and an anterior cone-shaped projection. The fluke possesses a powerful oral sucker at the end the anterior cone and a ventral sucker at the base of the cone which allow it to attach to the lining of the biliary ducts. Each worm possesses ovaries and testes which are highly branched and allow for individual flukes to produce eggs independently.

Fasciola hepatica is found on every continent with nearly 180 million people at risk and an estimated 2.4 million people already infected worldwide [43]. Prevalence is highest in areas where extensive sheep and cattle raising occurs and where dietary practices include the consumption of raw aquatic vegetables. In many locations such as Portugal, the Nile delta, northern Iran, parts of China, and the Andean highlands of Ecuador, Bolivia, and Peru, infections rates are high enough to make fascioliasis a serious public health concern [44].

The need for temperate, slow-moving or standing water in F. hepatica's life cycle and transmission had previously kept infection limited to populations within well-defined watershed boundaries [45]. Recently however, urbanization, migration, and development practices such as dam building and irrigation have increased the populations at risk and the incidence of human infection has increased significantly over the past 20 years (Chitsulo, Montresor, and Savioli).
Given the importance of serological methods for early detection of disease Fasciolosis and CANDIDIASIS in this article, the raw antigen excretion - secretion and somatic two species are compared with each other and lack of cross reactivity with antibodies or genes one species with antiserum other species examined in the ELISA test. According to the results presented in Table 1 antigen excretion - secretory with antiserum produced against antigens of excretory - secretory F. hepatica and C.albicans species have shown a strong reaction. Excretory-secretory antigens C.albicans with antiserum produced against the antigen excretory - secretory F. hepatica have strong reactions. According to Table 2 F. hepatica somatic antigens prepared with antiserum against somatic antigens C.albicans has shown strong response. C.albicans somatic antigens as well as with antiserum produced against F. hepatica somatic antigen have shown a strong reaction [7].

The results of this study show that antigenic materials F. hepatica and C.albicans have many similarities with each other and show a cross reaction in ELISA with each other. In other words, the antigens of both species can be developed for the detection of antibodies in the sera of immunized rabbits used against other species. These results are consistent with research findings Huang, These individuals also cross reactivity with antigens Excretory-secretory two an species has been reported in ELISA point [46].

CONCLUSION

According to the results of this Study further study on the identification, isolation and purification of materials Antigenic in any way in other species is absent Recommendation it is possible. In case access to these materials can be of serological methods for the detection of human or animal illness of any degree of sensitivity and specificity was higher.

REFERENCES