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Review

Incidence and the history of *Echinococcus granulosus* infection in dogs within the past few decades in Libya: A review

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Echinococcus granulosus **is a tiny tapeworm that parasitizes the small intestine of canids, mainly dogs, which act as definitive hosts for the parasite. Infected dogs are the main source of infection to humans and livestock which act as intermediate hosts resulting in hydatid disease condition. *E. granulosus* is widely distributed in many parts of the world, and is very common in North African countries. In Libya, the rate of infection with echinococcosis in dogs was reported to be lower than 7 to 80% in stray dogs, 34.8 to 60% in sheep/guard dogs and 7.7 to 21.6% in farm/house dogs. This data fulfills the world health organization (WHO) criteria and suggests that the incidence of infection with echinococcosis/ hydatidosis in some parts of the country can be reaching the level of hyper endemic. Diagnosis of echinococcosis in infected dogs can be performed by isolating the parasite from their faeces or from the contents of their small intestine after necropsy. Recent developments in immunodiagnostic assays for echinococcosis in dogs have been described. Public health and risk factors as well as ways of hydatid disease treatment and various control strategies, including the use of veterinary vaccines, have also been discussed.**

Key words: Dogs, *Echinococcus granulosus*, prevalence, diagnosis, treatment, risk factors

INTRODUCTION

Echinococcus granulosus is a tapeworm that causes a condition known as echinococcosis in dogs, and hydatid disease or hydatidosis in humans and other ruminant animals (Chhabra and Singla, 2009). The parasite has been reported to occur in many parts of the world, and is very common in some agricultural regions, particularly, Northern Africa, Southern South America, Europe, the Middle East, South-Western Asia, and Australia (Figure 1), (Eckert and Deplazes, 2004). In these areas, the infection rate with *E. granulosus* in dogs was reported to be between endemic and hyper endemic (Dakak, 2010). The parasite requires two mammalian hosts for

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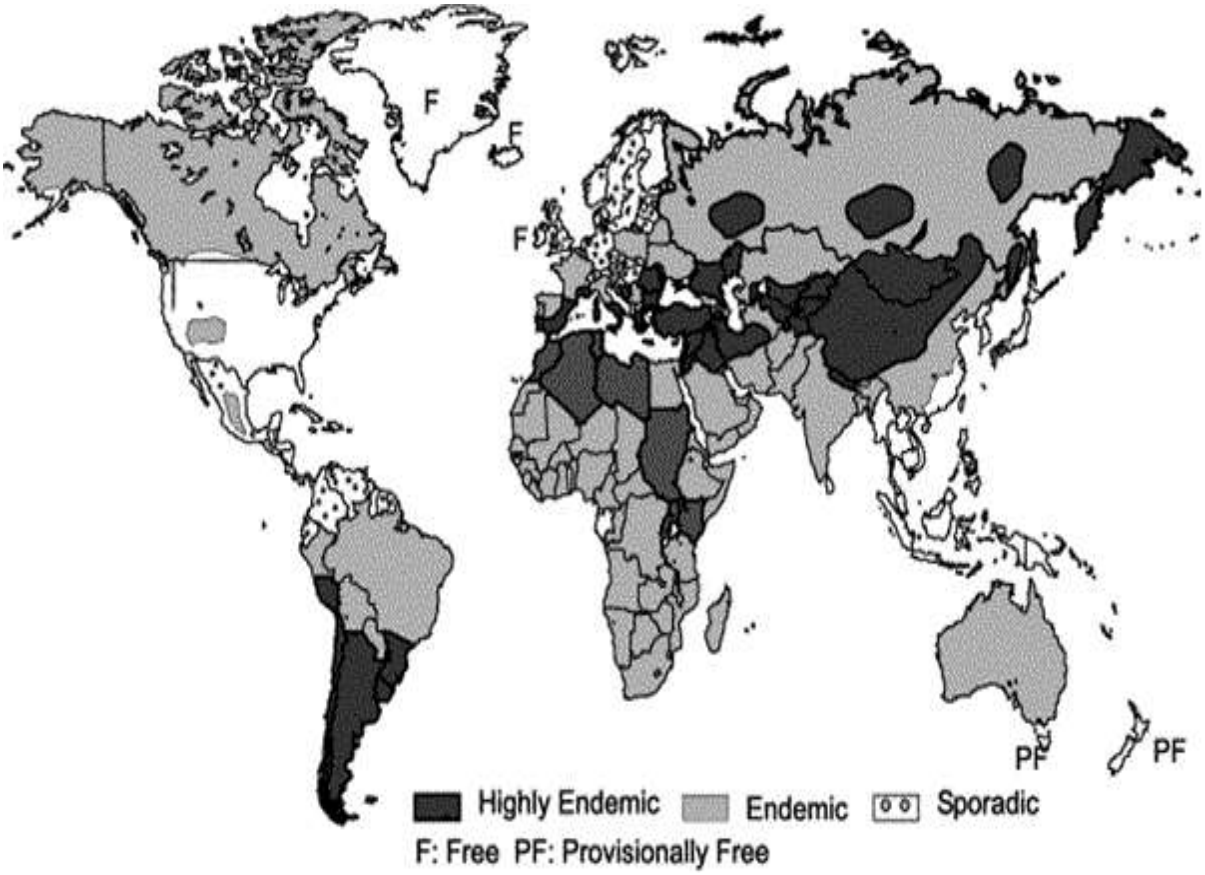

Figure 1. Worldwide distribution of *E. granulosus.* As seen from the map, echinococcosis infection in Libya in North Africa is highly endemic (adapted from Eckert and Deplazes, 2004).

completion of its life cycle; a definitive host, which is mainly carnivore for the adult stage and an intermediate host, which is mainly ungulates for the larval (hydatid cyst) stage (Figure 2). The life cycle of *E. granulosus* in Libya has a pastoral or domestic cycle in which dogs acquire the infection by eating the internal organs of infected sheep, goats, camels and cattle.

However, dogs in Libya are considered to be the main source of infection with cystic echinococcosis (CE) to various species of livestock as well as human. This cycle is potentially very important especially in areas where sheep farming is more common (Maureen, 2008). The adult form of the parasite is a minute white tapeworm, few millimeters long (3-7 mm) with three proglottids (segments) and some other features which help in species diagnosis morphologically (Thompson, 1995).

Due to the size of the parasite, dogs can carry hundreds or even thousands of them without showing any signs of illness at all. The parasites attach themselves to the wall of the small intestine of the definitive host using their hooks and in this place; they deposit a large number of eggs which are intermittently passed out in the host faeces. When the eggs are in the environment, they are dispersed by different means including wind, water, birds as well as through the fur of the infected dogs which is likely to become contaminated with the parasite eggs.

Eggs can also be found on the bodies of other animals sharing the same living environment, making them a source of transmission and distribution of the infection to humans and other ruminant animals. The intermediate hosts become infected with hydatid disease when they ingest the parasite eggs in their contaminated food or water. Once the eggs are ingested, they hatch in the duodenum releasing their embryos (the oncospheres) which subsequently penetrate the intestinal wall of the host entering the mesenteric vessels. They are then carried by the blood to the major filtering organs, mainly liver and lungs, but other sites may become involved, including the abdominal wall, brain, kidneys, bones, muscles and orbits (Polat et al., 2003; Bal et al., 2008).

The oncospheres requires about one year to transform and develop into full larval hydatid cysts with numerous tiny protoscoleces which are formed via asexual reproduction. Humans act as an intermediate hosts for *E. granulosus* and are infected when they accidentally ingest the parasite eggs from the definitive host by any way.

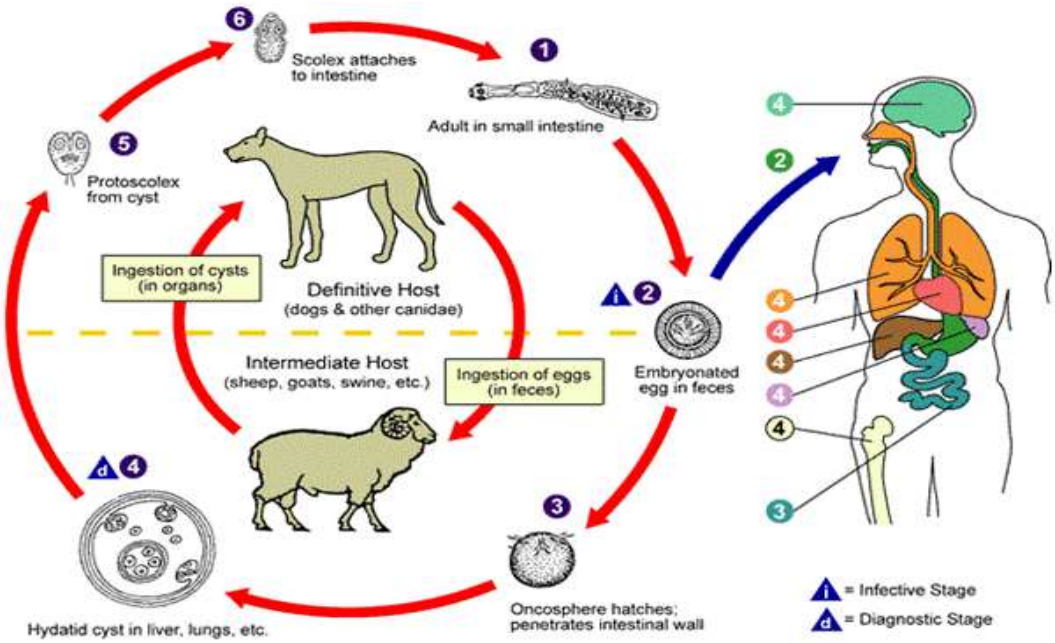

Figure 2. Illustration showing the life cycle of *E.granulosus* in both animals and humans. The life cycle of the parasite in animals involves six stages: 1) The adult worms resides attached to the bowel of the dogs. 2) Gravid segments release eggs that are passed out in the dog faeces. 3) The eggs are ingested by the ruminant animals and hatch in their bowels releasing oncospheres that invade the intestinal wall and travel through the circulating system to various organs of the host. 4). In the site, oncospheres develop into hydatid cysts producing protoscoleces and daughter cysts. 5) The ruminant infected organs ingested by dogs. 6) The protoscoleces attach to the intestinal wall of the dogs and start to develop gradually into adults in 32 to 80 days. The life cycle in humans: 2) Humans infected by eating food contaminated with the parasite eggs. 3) The ingested eggs hatch to release oncospheres in the small intestine. 4) Oncospheres migrate through the circulating system to different sites where they develop and produce hydatid cysts. (The image adapted from www.dpd.cdc.gov/dpdx).

The life cycle of the parasite is complete when dogs ingest hydatid cysts containing fully developed protoscoleces, which are subsequently released and attach themselves to the intestinal lining of the host. The protoscoleces start to develop into mature adult tapeworms within32- 80 days, depending on the species and the parasite strain. Humans are described as 'dead-end' hosts for the parasite, since the life cycle is usually completed when carnivores eat infected herbivores (McManus et al., 2003; Zhang et al., 2003).

However, it is mentioned in literature that, infected dead human bodies may contribute to complete the life cycle of the disease in some African villages where burial is not properly practiced and dogs can get access to those dead bodies (Macpherson et al., 1983). The research methodology consists of over 200 published articles on *E. granulosus* from different sources. The most appropriate publications covering the different sections in this paper were selected. This paper focuses mainly on the prevalence of *E. granulosus* infections in dogs in Libya since its discovery in 1961 to date and summarises the prospects of diagnosis, risk factors, treatment and the control and prevention strategies of the disease.

This study intends to provide base line epidemiological information on the incidence and the status of echinococcosis in dogs in Libya, and can be a source of information for the future studies.

Prevalence and the history of *Echinococcus granulosus* infection in dogs

According to scientists, the close relationship between humans and dogs started several thousands of years ago; this relationship facilitates the transmission of so many diseases including cystic hydatid disease.

Dogs in Libya, like any other country, can be classified into three groups, based on the type of their relationship to humans and other livestock: stray dogs, herding/sheep dogs and farm/house dogs. Stray dogs are the largest group in all urban and rural areas and they normally wonder freely during the night to scavenge on household waste materials, from which they may become infected with *E. granulosus* through eating dead animals and discarded offal contaminated with fertile hydatid cysts. These dogs are considered to be the main source of hydatid disease to human and livestock due to their

ability to spread the parasite eggs over a wide range of areas, especially where the suitable herbivores are grazing.

The second group is the herding/sheep dogs which are used for shepherding and guarding sheep. These dogs may become infected with *E. granulosus* as a result of eating offal contaminated with fertile hydatid cysts provided by their owners or discarded by other people. Herding/sheep dogs are considered to be a source of infection to livestock by contaminating grazing pastures and water pools used for grazing and drinking respectively by the animal flocks. The third group is the farm/house dogs which are used for guarding the farm belongings including animals and the house contents from thieves.These dogs become infected with *E. granulosus* through contaminated meat or offal provided mostly by their owners.

Echinococcosis was discovered in Libya for the first time in 1961, when heavy infections with *E. granulosus* were detected in 60% of shepherd dogs and 10% of town dogs (Cicogna, 1961). Data available on the prevalence of echinococcosis in dogs is scattered and inadequate. Three different studies have been conducted on the prevalence of echinococcosis in stray dogs between 1986 and 1990, and the obtained results after necropsy were 11.8, 40.3, and 36.8% (Packer and Ali, 1986; Gusbi, 1987a; Awan et al., 1990) respectively. In sheep and house dogs, the infection rate with echinococcosis was reported to be 34.8 and 7.7% respectively (Gusbi, 1987a).

The low rate of infection with *E. granulosus* in house dogs compared to the other two groups of dogs is probably due to the fact that these dogs are kept within premises and under control most of the time and they rarely get access to contaminated offal. Another study on the incidence of echinococcosis in dogs was carried out by Buishi et al. (2005), who reported that 25.8% of stray dogs and 21.6% of farm/house dogs were found to be harbouring the parasite. Using Kato thick smear technique, Ben-Musa and Sadek (2007) investigated 50 samples of faeces from street dogs and found that 58% of the examined specimens were positive for *E. granulosus*.

Moreover, a recent study was conducted by Gusbi (2010) examining 151 stray dogs at post mortem from 14 localities distributed all over the country. The obtained results showed that 27.8% of the examined dogs were infected with *E. granulosus*. This study also elucidated that, the infection rate was generally greater in the coastal areas of the country which was between 26 and 80%, and was even worse in Zawia, El-Khumes, Misrata, Sirt and Tubruk, where more than 50% of stray dogs were found to be harbouring the parasite.

In contrast, a previous study showed that in southern regions such as Sebha and El-Kufra, the infection rate in stray dogs was less than 7% (Gusbi, 1987a). This could simply be explained by the fact that huge numbers of abattoirs exist in the highly populated northern areas and are unfortunately lacking proper disposal of offal. Also moderate temperatures and the relatively high humidity in the northern areas may contribute significantly in prolonging the survival rate of *E. granulosus* eggs, thus allowing for an increased chance of disease transmission and vice versa in the southern areas (Wachira et al., 1991).

Furthermore, the differences in local traditions of slaughtering animals in houses during social occasions and celebrations, which involves the disposal of unwanted offal and remains by feeding it to domestic dogs, may contribute to the indicated variations.

Public health risks associated with *E. granulosus* infection in dogs

Despite the establishment of comprehensive and successful control programmes for CE, *E. granulosus* still continues to have a wide geographical distribution. This may lead to the re-emergence of the disease in many endemic regions worldwide, which would easily spread from endemic to non-endemic areas, causing severe public health problems and considerable economic losses (Craig et al., 2003; McManus et al., 2003).

Echinococcosis is a serious zoonosis in certain rural populations where there is close contact with domestic dogs and where human infection with CE is reported to be between > 1 and < 200 cases per 100,000 populations (OIE, 2008). In most endemic areas, where domestic dogs act as a definitive host for *E. granulosus*, identification of risk factors for canine infection can provide useful information on potential human risk and can be useful for designing and monitoring the parasite control schemes based on treatment of infection in dogs. Agricultural or stock-raising lifestyle, low socio-economic status, climate, bad hygiene, illegal or uncontrolled slaughter, as well as uncontrolled dog populations have all been reported to be risk factors (Cetinkaya et al., 2005; King and Fairley, 2010).

Laboratory workers, animal handlers, veterinarians, dog owners are more prone to infection with hydatid disease due to their direct contact with the parasite eggs. On the other hand, Muslim families who have the religious practice of keeping dogs away from homes and avoiding direct contact are reported to be at low risk of being infected with CE (Akalin et al., 2014). Eggs are usually shed to the environment and may therefore, contaminate vegetables, fruits, water, or stick to animals' fur and human hands. Great hygienic care is essential, especially careful hand washing practices which constitute an important preventive measure. In humans, hydatid cysts of *E. granulosus* are usually developed in organs such as liver and lungs, so the symptoms of infection with the disease will be liver or lungs deficiency, however, X-ray, ultrasound investigation and blood tests

should be undertaken regularly for those who are in contact with possible infected dogs. Significant risk factors for copro-positive owned dogs were found to be associated with non-restraint of dogs, in addition, people who do not de-worm their dogs, slaughter animals at home without proper veterinary inspection and have poor knowledge about the parasite transmission were also at high risk of acquiring human CE (Buishi et al., 2005).

Diagnosis of *E. granulosus* in dogs

Systematic diagnosis of *Echinococcus* infection in definitive hosts had always been an important component for establishing epidemiological parameters of echinococcosis and preventing human and livestock infection with CE (Sakai et al., 1995).

The problem of diagnosing *E. granulosus* in dogs has only been partially resolved, even after the introduction of biotechnology. It is more difficult to know when a dog is infected with *Echinococcus* parasites compared to the other cestods. This is due to the size of the parasite as well as their eggs, which can be easily missed out during faecal examinations and can be hard to differentiate them from *Taenia* eggs; however, this process still remains the most efficient way to detect the infection and should be performed regularly. Two major diagnostic methods have been used in dogs extensively. These are purgation with arecoline compounds and necropsy (Unruh et al., 1973; Craig et al., 1995; Eckert et al., 2001).

The purgation technique has been used in many control programmes all over the world in recent decades. The technique showed 100% specificity, but has certain limitations due to its poor sensitivity, as not all infected dogs respond to the purge and eliminate parasites. In addition to this, the technique is bio-hazardous, time consuming and must be administered by trained personnel (Craig et al., 1995; Eckert et al., 2001). Moreover, most of the epidemiological data and models have been developed from the results of this method (Torgerson et al., 2003). On the contrary, necropsy is the method of choice and is considered to be the more reliable tool for the diagnosis of the disease in dogs, but unfortunately has many limitations (Jenkins et al., 2000; Lopera et al., 2003).

Alternatively, immunodiagnostic techniques were used to detect specific antibodies or antigens in dogs. The detection of *E. granulosus* specific antigens in canine faeces was first reported by Babos and Nemeth (1962). During the last three decades, considerable progress has been achieved in various fields of echinococcosis research when several immunological and serological tests have evolved for the diagnosis of *Echinococcus spp.* in definitive hosts. Copro-antigen detection enzyme linked immunosorbent assay (cop-Ag-ELISA) test has been developed using polyclonal antibodies to *E.* granulosus excretory/secretory (ES) antigens, and appears to be valuable in detecting the infection in dogs with high specificity (96.5%) and sensitivity (87.5%) (Allan et al.,1992). Sandwich ELISA reported to be highly specific and capable in detecting immature and mature stages of *Echinococcus spp.* and this high specificity was found to be correlated to the worm burden and the duration of the infection (Craig et al., 1995; Ahmad and Nizami, 1998).

Some sandwich ELISA systems have been assessed for their ability to detect *E. granulosus* copro-antigens using monoclonal antibody produced against somatic extract of *Echinococcus multilocularis*. Although the test was very sensitive (100%) in naturally and experimentally infected animals, there were cases of cross-reactivity with *Taenia hydatigena* (Sakai et al., 1995; Malgor et al., 1997).

Overall, however, the test was the best laboratory-based test for ante mortem diagnosis of canine echinococcosis (Eckert et al., 2001). Parasite copro-antigens have been defined as parasite specific products in the faeces of the host that are amenable to immunological detection and are associated with the parasite metabolism (Allan et al., 2003).

Using copro-antigen sandwich ELISA, the sensitivity was between 83.33 and 100%, and the specificity was between 96.94 and 100% (Prathiush et al., 2008; Dalimi et al., 2010). Copro-ELISA test can also detect heat-stable antigens and has been used in a number of studies in the Middle East, Wales, Southern and Eastern Europe, and South America (Deplazes et al., 1992; Sakashita et al., 1995; Eckert et al., 2001).

The high sensitivity of monoclonal antibodies (MAb) to parasite specific antigens could increase the reliability of copro-antigen detection. Monoclonal antibodies for *E. granulosus* copro-antigen detection were produced namely, IgM murine monoclonal antibodies, EgC1 and EgC3, against E/S products of *E. granulosus* adult worms (Casaravilla et al., 2005).

Different studies from many countries suggest that copro-antigen ELISA is a valid test for detecting *E. granulosus* infection in living dogs. Thus, it is appropriate to apply this test in epidemiological studies (Magnaval et al., 2004; Buishi et al., 2005; Stefania et al., 2006; Kamiya et al., 2007; Zare-Bidaki et al., 2009). It would be useful to develop more specific techniques in cases where the presence of the parasite in the dog population is relatively low (Christofi et al., 2002), as well as for discrimination between dogs with *Echinococcus spp.* and those with other taeniid infections.

Copro-DNA-polymerase chain reaction (Copro-DNA-PCR) technique has been developed, and is only available for a limited number of species or genotypes in particular *E. multilocularis* and *E. granulosus* sheep strain (Craig et al., 2003; Mathis and Deplazes, 2006). Bretagna et al. (1993) was the first who developed a species-specific Copro-DNA-PCR for *E. multilocularis* and the technique showed 100% for both specificity and

sensitivity, but the later can vary depending on worms quantity and maturity (Mathis and Deplazes, 2006). Copro-DNA-PCR could be improved if the parasite eggs are concentrated by using a process called sequential sieving and zinc chloride flotation (Mathis et al., 1996).

A PCR test developed by Cabrera et al. (2002) showed high levels of specificity and sensitivity for the identification of *E. granulosus* eggs from a contaminated environment. However, it is clear that the test did not cross-react with *E. multilocularis* but shared similar genetic sequences to other *Echinococcus spp*. such as *Echinococcus oligarthrus* and *Echinococcus vogeli*. A Copro-DNA-PCR assay developed by Stefanic et al. (2004) for detection of *E. granulosus* sheep strain (G1) showed 100% specificity against other *Echinococcus spp*. including *E. multilocularis* and *E. vogeli*. The PCR test used by Abbasie et al. (2003) gave 100% sensitivity and specificity using DNA samples extracted from 0.3 ml of faeces from 34 infected and 18 non-infected dogs, and the test gave a positive result even with a small number of *E. granulosus* eggs in the sample. For field application, the cop-Ag-ELISA has the potential for replacing necropsy examinations. The cop-PCR is a valuable method for the confirmation of positive copro-antigen results and the diagnosis of individual animals (Deplazes and Eckert, 1996). It is indeed considered to be the most specific diagnostic technique (Mathis and Deplazes, 2006).

Furthermore, when using specific primer along with cop-PCR, the *Echinococcus* infection can be diagnosed up to species level, with a specificity of 100% (Stefanic et al., 2004; Dinkel et al., 2004).

Treatment of echinococcosis in dogs with reference to human cystic hydatidosis

Until recently, surgery was the only option for treatment of hydatid disease in humans. However, medication and other surgical techniques (aspiration) are currently widely in use and can replace the need for surgical removal of hydatid cysts (Polat et al., 2002).

Nevertheless, in some cases, a surgery may be necessary, along with medication, to prevent the cyst from growing back. It has been agreed that an image-based, stage-specific approach is helpful in determining the choice of human CE treatment, whether it be percutaneous treatment, surgery, and anti-infective drug treatment or watch and wait (Brunetti et al., 2010).

Puncture aspiration, re-aspiration, injection and chemotherapy are also available for treatment of CE (Pawłowski et al., 2001). Percutaneous drainage has been widely used as an alternative to surgery in the treatment of hydatid cysts. Unlike surgical procedures, which normally start with the inactivation of the cyst contents followed by removal of all cyst components (Yorganci and Sayek, 2002; Menezes da Silva, 2003), The percutaneous drainage method does not involve the removal of the cyst membrane which is composed of laminated and germinative layers (Yorganci and Sayek, 2002). Percutaneous drainage may include puncture, aspiration of cyst contents, injection of scolicidal agents and finally re-aspiration of the injected fluid, as described by Ben-Amor et al. (1986), or by catheterization, as described by Akhan and Özmen, (1999).

Chemotherapy became a treatment option for hydatid disease decades ago when new anti-helminthic drugs were introduced. Benzimidazole carbamates were shown to kill the entire larval stage of the parasite by inhibiting the formation of microtubules, and thus destructing the uptake of glucose and interfering with the homeostasis of the parasite (Lacey, 1990), whereas praziquantel was found to be effective on protoscoleces (Heath and Chevis, 1974; Schantz et al., 1982).

Continuous or irregular treatment with albendazole is recommended for a period of up to 6 months, and to increase the efficacy of the treatment, praziquantel should be used, particularly in the case of cyst spillage (Teggi et al., 1993). It has been reported that the infection caused by adult worms in dogs can be successfully treated with praziquantel and it is advisable to confine dogs and/or use purgatives to facilitate the collection and disposal of infected faeces.

Most studies indicate that the effectiveness of albendazole as measured by the disappearance of the cysts, is generally less than 30% under ideal circumstances. However, 40 to 50% of cysts showed some response during the course of therapy such as shrinkage in their size or detachment of their components from the cyst wall. To increase its efficacy, albendazole must be taken daily for 4 to 6 weeks and should be repeated two or three times more. Additionally, using albendazole before and after operation was found to decrease the viability of cysts at the time of surgery, as well as significantly reducing the chances of cyst recurrence (Arif et al., 2008).

Oxfendazole is a benzimidazole drug which has been used in veterinary medicine to control nematode infections and was found to be effective against the intestinal stage of *E. granulosus,* as well as other cestodes in the gastrointestinal tract, thus could be used to treat infections in dogs, the principal reservoir for human infection (Gemmell et al., 1979).

Gonzales et al. (1996) examined the effect of the drug on the tissue stage of tapeworm infections and found that a single dose of 30 mg/kg of body weight of oxfendazole in pigs completely eliminated all tissue cysts of *Taenia solium*, a medically important human tapeworm. Nevertheless, hydatid cysts are much larger than and structurally different from the cysts of cysticercosis, suggesting the possible use of oxfendazole for the treatment of hydatid disease. Further studies carried out by Blanton et al., (1998) and Njoroge et al., (2005) used the same dose of oxfendazole on other animal species.

The results obtained after post mortem investigation showed that 97% of cysts from sheep and 93.3% of cysts from goats contained dead or absent protoscoleces, compared to 28 and 27.3% of cysts from untreated control sheep and goats respectively. In addition, 53% of cysts from treated animals were found to be heavily degenerated and even with the potentially viable cysts, there was evidence of severe damage to the wall, severe disorganization of the adventitial layer with invasion of inflammatory cells and in some cases frank necrosis with no apparent adventitial layer (Blanton et al., 1998).

More evaluation on oxfendazole was carried out against CE in sheep and the obtained results showed that, the number of fertile cysts decreased, the number of degenerated cysts increased and it was more efficacious against lungs and liver cysts at 49.6 to 61.2% and 91.8 to 100% respectively (Gavidia et al., 2009).

Based on the reported results, oxfendazole appears to be a promising alternative drug for the treatment of CE and may potentially become the drug of choice for the treatment of human hydatid disease in the near future. However, with poor response to most chemotherapeutic agents, cystic hydatidosis remains a primarily surgical disease and the importance of using chemotherapy lies in the prophylaxis against spillage during surgery, treatment of none operated cases and for use in areas where adequate surgical facilities are unavailable.

Control and prevention of echinococcosis transmission

Cystic hydatidosis continues to be a strong cause of morbidity and mortality in many parts of the world, however, complete eradication of CE is difficult to obtain and by using current control options to achieve such a goal will take several years of continuing attempts (Craig et al., 2007).

Control of unilocular hydatidosis is based on breaking the cycle of infection, either by preventing dogs from consuming infected organs of intermediate hosts or by preventing intermediate hosts from ingesting eggs present in dogs' faeces and treating infected dogs with effective cestocides, especially in urban environments. Cystic hydatid disease in humans was found to be caused by different genotypes of *E. granulosus* subspecies. Such genotypes include *for example, sensu stricto* (G1-G3), *equinus* (G4), *ortleppi* (G5) and *canadensis* (G6-G10) (Thompson, 2008).

In Libya, most human cases of CE are caused by sheep strain G1, cattle strain G5 and camel strain G6. These intermediate hosts are the most common reared animals in the country (Abushhiwa et al., 2010). Dogs are the most essential part for hydatid disease transmission to humans and other ruminant animals; however, vaccination of dogs provides a very practical and cost-effective Prevention strategy.

A study by Zhang et al. (2006) indicated that vaccination of these animals with soluble native proteins obtained from protoscoleces of *E. granulosus* promotes significant suppression of worm growth and eggs production. In addition, control strategies need to focus on careful analysis of the local situations such as the cycle, ecology, and ethology of the animal hosts, as well as behavioural characteristics of the population at risk. It is most important to use newly developed tools such as imaging, molecular biology and immunology in both human and animals in any successful control programme. Moreover, anti-parasitic treatment, control of the definitive hosts, control of slaughtering, vaccination of the intermediate hosts, health education are also considered to be essential elements in any control programme (Ito et al., 2003).

As it is difficult to completely prevent the exposure to *Echinococcus* eggs from wild animals, food safety precautions combined with good hygiene can be helpful. All fruits and vegetables, especially those picked up from the wild, should be cleaned thoroughly with water to ensure the removal of the parasite eggs, if any. People who handle pets, or are involved in farming, gardening or preparing food, should wash their hands carefully before eating.

Furthermore, fences should be built around vegetable and fruit gardens to keep dogs and other canids away. Untreated water from sources such as lakes may also contain *Echinococcus* eggs and should therefore, be avoided. Unfortunately, over the past decades, there has been no CE control programme in Libya, but the high incidence of the disease in humans (1.4 to 2%) (Shambesh et al., 1999), and the high prevalence of hydatidosis in livestock, which has been reported to be 1.6 to 40% in sheep, 5.6 to 70% in goats, 2.7 to 56% in cattle, and 2.7 to 48% in camels (Ibrahem et al., 2016), suggests the need for an extensive control programme.

A control programme is most effective when implemented on a community or county-wide basis and must include the de-worming of all dogs, especially those with possible access to livestock offal, and this must be repeated at any time after any possible exposure. In endemic areas, where echinococcosis is considered to be a public health concern, dogs should be dosed with praziquantel every 6 weeks. The correct disposal of dead animals or animal viscera, elimination of stray dogs, keeping dogs away from children play grounds, personal hygiene (hand washing) are all essential tools in reducing the chances of the disease endemicity.

Despite the available epidemiological information on echinococcosis/hydatidosis in Libya, indicating that the disease is a public health concern, no effective control programme is currently in place. There is evidence suggesting that the incidence of echinococcosis and hydatidosis may have increased in the country during the last few years, due to the major social and political changes that affected veterinary and public health

services following the collapse of the country government. Conducting screening surveys, using serological tests, may help in detecting early infections particularly in high risk groups.

CONCLUSION

Echinococcosis is a prevalent disease in all dog groups in Libya, with variable rates of infection (< 7 to 80% in stray dogs, 34.8 to 60% in sheep or guard dogs and 7.7 to 21.6% in farm or house dogs). Attempts to develop techniques with high specificity and sensitivity for the diagnosis of echinococcosis and hydatidosis are important in understanding the disease's epidemiology, especially in areas where CE is recently discovered. Control of hydatidosis is less effective without the support of dog-owners and this can only be achieved through increasing education and raising community awareness of the disease (Heath et al., 2006). High precautions should be taken in consideration regarding the risk factors influencing the transmission and spread of the disease in areas where the disease is recognised. In areas where home slaughter is practiced, dosing of dogs with a suitable taeniacide will be an important component in the hydatid control programme (Watson-Jones and Macpherson, 1988). In developing countries like Libya, imposing strict measures on offal disposal in abattoirs will certainly reduce disease transmission (Ito et al., 2003).

Conflict of Interests

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REFERENCES

Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, Craig PS, Hamburger J (2003). Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. Am. J. Trop. Med. Hyg. 69:324-30.

Abushhiwa MH, Nolan MJ, Jex AR, Campbell BE, Jabbar A, Gasser RB (2010). Genetic classification of *E. granulosus* cysts from human, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. Mol. Cell Probes 24:346-351.

Ahmad G, Nizami WA (1998). Copro-antigens: Early detection and suitability of an immunodiagnostic method for echinococcosis in dogs Vet. Parasitol. 77:237-244.

Akalin S, Kutlu SS, Caylak SD, Onal O, Kaya S, Bozkurt AI (2014). Seroprevalence of human cystic echinococcosis and risk factors in animal breeders in rural communities in Denizli, Turkey. J. Infect. Dev. Count. 8: 1188-1194.

Akhan O, Özmen MN (1999). Percutaneous treatment of liver hydatid cysts. Eur. J. Radiol. 32:76-85.

Allan JC, Craig PS, Garcia Noval J, Mencos F, Liu D, Wang Y, Wen H, Zhou P, Stringer R, Rogan MT, Zeyhle E (1992). Copro-antigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitol. 104:347-356.

Allan JC, Wilkins PP, Tsang VC, Craig PS (2003). Immunodiagnostic tools for taeniasis. Acta Trop. 87:87-93.

Arif SH, Shams-ul-Bari, Wani NA, Zargar SA, Wani MA, Tabassum R, Hussain Z, Baba AA, Lone RA (2008). Albendazole as an adjuvant to the standard surgical management of liver hydatid cyst. Int J. Surg. 6:448-451.

Awan MAQ, Gusbi AM, Beesley WN (1990). *Echinococcus* in Libya. III. Further studies of *E. granulosus* in dogs. Ann. Trop. Med. Parasitol. 48:473-475.

Babos S, Nemeth I (1962). Az echinococcis szerodiagnoztikajanak kerderehez. Mag. Allat. Lap. 17:58-60.

Bal N, Nazim EK, Rabia A, Ali E, Fazilet K (2008). Uncommon locations of hydatid cysts. Saudi Med. J. 29:1004-1008.

Ben-Amor N, Gargouri M, Gharbi HA, Golvan YJ, Ayachi K, Kchouck H (1986). Treatment of hepatic hydatid cyst in sheep by echographic puncture. Tunis. Med. 64:325-331.

Ben-Musa NA, Sadek GS (2007). Prevalence of echinococcosis in street dogs in Tripoli district, Libya. J. Egypt Soc. Parasitol. 37:793-800.

Blanton R, Wachira TM, Zeyhle E, Njoroge EM, Magabo J, Schantz PM (1998). Oxfendazole treatment for cystic hydatid disease in naturally infected animals. Am. Soc. Microbiol. 42:601-605.

Bretagna S., Guilloun JP, Morand M, Houin R (1993). Detection of *Echinococcus multilocularis* DNA in fox faeces using DNA hybridisation. Parasitol. 106:193-199.

Brunetti E, Kern P, Vuitton DA (2010). Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 114:1-16.

Buishi IE, Njoroge EM, Bouamra O, Craig PS (2005). Canine echinococcosis in northwest Libya: Assessment of coproantigen ELISA, and a survey of infection with analysis of risk-factors. Vet. Parasitol. 130: 223-232.

Cabrera M, Canova S, Rosenzvit M, Guarnera E (2002). Identification of *Echinococcus granulosus* eggs. Diagn. Microbiol. Infect. Dis. 44:29-34.

Casaravilla C, Malgor R, Rossi A, Sakai H, Nonaka N, Kamiya M, Carmona C (2005). Production and characterisation of monoclonal antibodies against excretory/secretory products of adult *Echinococcus granulosus*, and their application to coproantigen detection. Parasitol. Int. 54:43-49.

Cetinkaya Z, Ciftci IH, Demirel R, Altindis M, Ayaz E (2005). A seroepidemiologic study on cystic echinococcosis in Midwestern region of Turkey. Saudi Med. J. 26:350.

Chhabra MB and Singla LD (2009). Food-borne parasitic zoonoses in India: Review of recent reports of human infection. J. Vet. Parastol. 23:103-110.

Christofi G, Deplazes P, Christofi N, Tanner I, Economides P, Eckert J (2002). Screening of dogs for *E. granulosus* coproantigen in a low endemic situation in Cyprus. Vet. Parasitol. 104:299-306.

Cicogna D (1961). L'echinociccosi in Tripolitania. Boll. San. Del. Trip. pp. 2:3.

Craig PS, Gasser RB, Parada L, Caprera P, Parietti S, Brgues C, Acuttis A, Agulla J, Snowden K, Paolillo E (1995). Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody test with arecoline purgation in Uruguay. Vet. Parasitol. 56:293-301.

Craig PS, McManus DP, Lightwolers MW, Chabalgoity JA, Garcia HH, Gavidia CM, Gilman RH, Gonzalez AE, Lorca M, Naquira C, NietoA, Schantz PM (2007). Prevention and control of cystic echinococcosis. Latent Infect. Dis. 7:385-394.

Craig PS, Rogan MT, Campos-Ponce M (2003). Echinococcosis: disease, detection and transmission. Parasitol. 127:Suppl S5-20.

Dakak A (2010). Echinococcosis/hydatidosis: A severe threat in Mediterranean countries. Vet. Parasitol. 174:2-11.
Dalimi A, Motamedi GHR, Syavashi M, Paykari H, Hablolvarid MH (2010). Detection of *Echinococcus granulosus* infection in dogs using coproantigen enzyme linked immunosorbent assay. Arch. Razi. Inst. 65:9-14.
Deplazes P, Eckert J (1996). Diagnosis of the *Echinococcus multilocularis* infection in final hosts. Appl. Parasitol. 37:245-252.
Deplazes P, Gottstein B, Eckert J, Jenkins DJ, Ewald D, Jimenez-Palacios S (1992). Detection of *Echinococcus* coproantigens by enzyme linked immunosorbent assay in dogs, dingoes and foxes. Parasitol. Res. 78:303-308.
Dinkel A, Njoroge EM, Zimmermann A, Walz M, Zeyhle E, Elmahdi EI, Mackenstedt U, Romig T (2004). A PCR system for detection of species and genotypes of the *Echinococcus granulosus*-complex, with reference to the epidemiological situation in eastern Africa, Int. J. Parasitol. 34:645-653.
Eckert J, Deplazes P (2004). Biological, epidemiological and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev. 17:107-135.
Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers M, Meslin FX, Pawłowski ZS (2001). Echinococcosis in animals: clinical aspects, diagnosis and treatment. WHO/OIE Manual on echinococcosis in humans and animals: a public health problem of global concern. pp. 72-99.
Gavidia CM, Gonzalez AE, Lopera L, Jayashi C, Angelats R, Barron EA, Ninaquispe B, Villarrea L, Garcia HH, Verastegui MR, Gilman RH (2009). Evaluation of nitazoxanide and oxfendazole efficacy against cystic echinococcosis in naturally infected sheep. Am. J. Trop. Med. Hyg. 80: 367-372.
Gemmell MA, Johnstone PD, Oudemans G (1979). The effect of oxfendazole on *Echinococcus granulosus* and *Taenia hydatigena* infections in dogs. Res. Vet. Sci. 26:389-390.
Gonzales AE, Garcia HH, Gilman RH, Gavidia CM, Tsang VCW, Bernal T, Falcon N, Romero M, Lopez-Urbina MT (1996). Effective, single-dose treatment of porcine cysticercosis with oxfendazole. Am. J. Trop. Med. Hyg. 54:391-394.
Gusbi AM (1987a). Echinococcosis in Libya. I. Prevalence of *Echinococcus granulosus* in dogs with particular reference to the role of the dogs in Libyan Society. Ann. Trop. Med. Parasitol. 81:29-34.
Gusbi AM (2010). The prevalence of *E. granulosus* infection in dogs and the parasitic risk in Libya. 4th Sci. Congr. Egypt. Soc. Anim. Manag. 112-119.
Heath D1, Yang W, Li T, Xiao Y, Chen X, Huang Y, Yang Y, Wang Q, Qiu J (2006). Control of hydatidosis. Parasitol. Int. 55:247-252.
Heath DD, Chevis RAF (1974). Mebendazole and hydatid cysts. Lanc. 2:218-219.
Ibrahem, MM, Ibrahem WM, Abdorrahem MM, Ibrahem KM (2016). Livestock Hydatid Disease (Cystic Hydatidosis) in Libya: A review. Am. J. Anim. Vet. Sci. 11:70-84.
Ito A, Urbani C, Jiamin Q, Vuitton DA, Dongchuan Q, Heath DD, Craig PS, Zheng F, Schantz PM (2003). Control of echinococcosis and cysticercosis: A public health challenge to international cooperation in China. Acta Trop. 86:3-17.
Jenkins D J, Fraser A, Bradshaw H, Craig PS (2000). Detection of *Echinococcus granulosus* coproantigens in Australian canids with natural or experimental infection. J. Parasitol. 86:140-145.
Kamiya M, Lagapa JT, Ganzorig S, Kobayashi F, Nonaka N, Yuzaburo O (2007). Echinococcosis risk among domestic definitive hosts, Jap. Emerg. Infect. Dis. 13:346-47.
King CH, Fairley JK (2010). Cestodes (Tapeworms). In. Mandell GL, Bennett JE, Dolin R (Eds). Principles and Practice of Infectious Diseases. 7th edition. Philadelphia, Churchill Livingstone. pp. 3607-3616.
Lacey E (1990). Mode of action of benzimidazole. Parasitol. Tod. 6:112-115.
Lopera L, Moro P, Chavez A, Montes G, Gonzales A Gilman R (2003). Field evaluation of a coproantigen enzyme linked immunosorbent assay for diagnosis of canine echinococcosis in a rural Andean village in Peru. Vet. Parasitol. 117:37-42.
Macpherson CNL, Karstad L, Stevenson P, Arundel JH (1983). Hydatid disease in the Turkana District of Kenya. III. The Significance of Wild Animals in the Transmission of *Echinococcus granulosus*, with particular reference to Turkana and Masailand in Kenya. Ann. Trop. Med. Parasitol. 77:66-73.
Magnaval JF, Boucher C, Morassin B, Raoul F, Duranton C, Jacquiet P, Giraudoux P, Vuitton DA, Piarroux R (2004). Epidemiology of alveolar echinococcosis in southern Cantal, Auvergne region, France. J. Helminthol. 78:237-242.
Malgor R, Nonaka N, Basmadjian I, Sakai H, Carambula B, Oku Y, Carmona C, Kamiya M (1997). Coproantigen detection in dogs experimentally and naturally infected with *Echinococcus granulosus* by a monoclonal antibody-based enzyme linked immunosorbent assay. Int. J. Parasitol. 27:1605-1612.
Mathis A, Deplazes P (2006). Copro-DNA tests for diagnosis of animal taeniid cestodes. Parasitol. Int. 55:87-90.
Mathis A, Deplazes P, Eckert J (1996). An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. J. Helminthol. 70:219-22.
Maureen A (2008). More about *Echinococcus* tape worm in dogs. Worms & Germs Blog, Ont. Vet. Coll., Centre for Public Health and Zoonosis.
McManus DP, Zhang W, Li J, Bartley PB (2003). Echinococcosis. Lanc. 362:1295-1304.
Menezes da Silva A (2003). Hydatid cyst of the liver: Criteria for the selection of appropriate treatment. Acta Trop. 85: 237-242.
Njoroge E, Mbithi P, Wachira T, Gathuma J, Gathura P, Maitho TE, Magambo J, Zeyhle MSE (2005). Comparative study of albendazole and oxfendazole in the treatment of cystic echinococcosis in sheep and goats. Int. J. Appl. Res. Vet. Med. 3:97-101.
Office International des Epizootics (OIE) (2008). Echinococcosis/ hydatidosis. Terrestrial manual.
Packer DE, Ali TM (1986). *Echinococcus granulosus* in dogs in Libya. Ann. Trop. Med. Parasitol. 80:137-139.
Pawloeski ZS, Eckert J, Vuiton DA, Ammann RW, Kern P, Craig PS, Dar KF, Rosa F, de Filice C, Gottesein B, Grimm F, Macpherson CNL, Sato N, Todorov T, Uchino J, Sinner W, von Wen H (2001). Echinococcosis in humans: clinical aspects, diagnosis and treatment. In. Eckert J, Gemmell MA, Pawloeski ZS, (Eds). Manual on echinococcosis in humans and animals: A public health problem of global concern. WHO/OIE. pp: 20-66.
Polat KY, Balik AA, Oren D (2002). Percutaneous drainage of hydatid cyst of the liver: long-term results. HPB. 4:163-166.
Polat P, Mecit K, Fatih F, Selami S, Melike BK, Adnan O (2003). Hydatid Disease from Head to Toe 1. Radiographics 23(2):475-494
Prathiush PR, Placid ED, Ananda KJG (2008). Diagnosis of *Echinococcus granulosus* infection in dogs by a coproantigen sandwich ELISA. Vet. Arch. 78:297-305.
Sakai H, Malgor R, Basmadjian I, Gallardo R, Carmona C, Sato H, Oku Y, Kamiya M (1995). An enzyme linked immunosorbent assay (ELISA) for the detection of *Echinococcus granulosus* coproantigen in dogs. Japan J. Parasitol. 44:453-461.
Sakashita M, Sakai H, Kohno H, Ooi V, Oku V, Vagì K, Ito M, Kamiya M (1995). Detection of *Echinococcus multilocularis* coproantigens in experimentally infected dogs using murine monoclonal antibody against adult worms. Jap. J. Parasitol. 44:413-420.
Schantz PM, Van den Bossche H, Eckert J (1982). Chemotherapy for larval echinococcosis in animals and humans: Report of a workshop. Z. Parasit. 53:5-26.
Shambesh MK, Craig PS, Macpherson CNL, Rogan MT, Gusbi AM, Eechtuish FE (1999). An extensive ultrasound and serologic study to investigate the prevalence of human cystic echinococcosis in northern Libya. Am. J. Trop. Med. Hyg. 60:462-468.
Stefania S, Avram E, Cozma V (2006). Detection of *Echinococcus* coproantigens by enzyme linked immunosorbant assay in dogs from the northwest of Romania. Bull. USAMV-CN 63:395-398.
Stefanic S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR, Mathis A (2004). Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* (sheep strain) in naturally infected dogs. Parasitol. Res. 92: 347-351.
Teggi A, Lastilla MG, De Rosa F (1993). Therapy of human hydatid disease with mebendazole and albendazole. Antimicrob. Agents Chemother. 37:1679-1684.
Thompson RCA (2008). The taxonomy, phylogeny and transmission of

Echinococcus. Exp. Parasitol. 119:439-446.
Thompson RCA (1995). Biology and systematics of *Echinococcus.* In: Thompson RCA, Lymbery AJ (Eds.). The biology of Echinococcus and hydatid disease. Cab International. pp. 1-50.
Torgerson PR, Shaikenov BS, Rysmukhambetova AT, Abdybekova AM, Usenbayev AE, Baitursi nov KK (2003). Modelling the transmission dynamics of *Echinococcus granulosus* in rural Kazakhstan. Vet. Parasitol. 114:143-153.
Unruh DHA, King JE, Eaton RDP, Allen JR (1973). Parasites of dogs from Indian settlements in northwestern Canada: A survey with public health implications. Can. J. Comp. Med. 37:25-32.
Wachira TM, Macpherson CNL, Gathuma JM (1991). Release and survival of *Echinococcus* eggs in different environments in Turkana, and their possible impact on the incidence of hydatidosis in man and livestock. J. Helminthol. 65:55-61.
Watson-Jones DL, Macpherson CNL (1988). Hydatid disease in the Turkana district of Kenya. VI. Man-dog contact and its role in the transmission and control of hydatidosis amongst the Turkana. Ann. Trop. Med. Parasitol. 82:343-356.
Yorganci K, Sayek I (2002). Surgical treatment of hydatid cysts of the liver in the era of percutaneous treatment. Am. J. Surg. 184:63-69.
Zare-Bidaki M, Mobedi I, Naddaf SR, Kia EB, Mahmoudi M, Piazak N, Nekouie H, Sadeghieh Ah ari S, Habibzadeh Sh, Siavashi M (2009). Prevalence of *Echinococcus spp*. infection using coproantigen ELISA among canids of Moghan plain, Iran. Iran J. Public Health 38:112-118.
Zhang W, Li J, McManus PD (2003). Concepts in immunology and diagnosis of hydatid disease. Clin. Microbiol. Rev. 16:18-36.
Zhang W., Zhang Z., Shi B, Li J, You H, Tulson G, Dang X, Song Y, Yimiti T, Wang J, Jones MK, McManus DP. (2006). Vaccination of dogs against *Echinococcus granulosus* the cause of cystic hydatid disease in humans. J. Infect. Dis.194:966-974.

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Prevalence of endo-parasites in donkeys and camels in Karamoja sub-region, North-eastern Uganda

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Donkeys (*Ass-Equus-assinus*) and camels (*Camelus dromedarius*) in Uganda are mainly owned by low income earners and peasant farmers, mainly in the semi-arid North-eastern Uganda in Karamoja and Sebei sub-regions. The animals however, seem to receive little or relatively no veterinary care. This study was carried out in Moroto and Amudat districts, Karamoja sub-region, Uganda in March 2016. Faecal samples of 110 randomly selected camels and donkeys of all age and sex were collected directly from their rectum. The faecal samples were examined by flotation method, sedimentation technique, McMaster egg counting technique and faecal culture to identify and determine the burden of parasites in different age groups. Lungworms ovaculture revealed *Dictyocaulus cameli* (29.3%) of camels and *Dictyocaulus arnfieldi* (15.4%) of donkeys. Cestode eggs detection revealed family *Anoplocephalidae* which includes seven species of cestodes identifiable as eggs in faeces or as adults in the gastro-intestinal tract of camels (18.3%) and donkeys (15.4%). Coccidia species included *Eimeria cameli* (11%) and *Eimeria Leuckarti* (3.85%) in camels and donkeys, respectively. Trematodes were detected in 5 camels as *Fasciola gigantica*. Overall, EPG count was observed for strongyles at 58.5% in camels and 42.3% in donkeys. The results of this study will provide insights into the health of donkeys and camels in Uganda and provide a way forward to their veterinary care and management for improved production and productivity.

Key words: Gastrointestinal parasites, donkey, camels, Karamoja, Uganda.

INTRODUCTION

Donkeys and camels are subject to severe work and in bondage, working in extremely strenuous conditions, over laden, underfed and ill-used especially for draught purposes. They are sore footed, constantly burdened by parasites (Atawalna et al., 2015; Tsegaye and Chala, 2015; Ismail et al., 2016; Anvari-Tafti et al., 2013), with little or no veterinary care. An attempt to study parasitism in these species has only been recent.

Parasitic helminthes are one of the most common factors that constrain the health and working performance of camels and donkeys worldwide. Parasites cause various degrees of damage depending on the species and number present, nutritional and the immune status of the animal (Sumbria et al., 2014; Sumbria and Singla,

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2015). Infection by endo-parasites in camels and donkeys are responsible for problems including poor body condition, reduced power output, diarrhea, colic, emaciation, impaired growth, poor reproductive performance, short lifespan and predisposition to other infectious diseases (Ayele, 2006; Fikru et al., 2005; Getachew et al., 2010; Yoseph et al., 2005).

The prevalence, species composition and epidemiology of helminthes affecting donkeys and camels have not been previously investigated in Uganda. Donkeys and camels are used in arid and mountain villages, both as saddle animals and for carriage. In camels and donkeys, nematodes are seen to be most prevalent, while cestodes and trematodes are less occurring (Arslan and Umur, 1998; Bakirci et al., 2004; Demir et al., 1995). *Eimeria* species can also be encountered (Arslan and Umur, 1998; Bakirci et al., 2004; Ozer and Küçükerden, 1992).

Nematode infection was the most prevalent challenge in these species. A heavy internal parasite burden can adversely affect the health of donkeys and camels particularly when called upon to work and as it is often the case, is undernourished and stressed (Sonja et al., 2000).

The present study was therefore designed to generate baseline data on the prevalence and species composition of helminth parasites of donkeys and camels in Uganda.

MATERIALS AND METHODS

Faecal samples were collected from Karamoja sub-region in two districts namely: Moroto: N 2° 31' 41.604", E 34° 39' 28.794" and Amudat: N 1° 47' 29.841", E 34° 54' 23.583" districts, Uganda. The study was conducted in March 2016. The camels and donkeys were classified as: infant, juvenile, sub-adult and adult. Male and female animals were sampled (Table 1).

Karamoja sub-region is a semi-arid region, the livelihoods of the people depend on pastoralism. Cattle, sheep and goat are kept in large numbers for meat, milk, blood, dowry, prestige. Donkeys and camels are kept for draught power, meat and milk. Faecal samples were collected directly from the rectum of the animal using a gloved hand, into polyethene bags, labeled using details such as source, age, sex, species and general condition of animal for easy identification, cold chain was maintained in the field and the samples were hurriedly taken to the parasitology laboratory for analysis to avoid larval development.

Samples were kept in refrigerator at 4°C if immediate processing was not possible, but it had been processed within 48 h or as soon as possible. Direct faecal smear, sedimentation and floatation techniques were the utilized parasitological techniques to identify the eggs in faeces and examined microscopically (10 and 40×) for presence of parasite ova following their procedures (Forety, 2001). Identification of the eggs was made on the basis of their morphology (Soulsby, 1982). Quantitative faecal examination was performed by using McMaster technique (Gordon and Whitlock, 1939) to determine the number of egg per gram of faeces (EPG) and performed according to the procedure described by Urquhart et al. (1996). Faecal culture using Baermann technique to determine lungworm larvae, *Dictyocaulus arnifieldi* and *Dictyocaulus cameli* was also undertaken. Level of infection was extrapolated from infection severity index (Soulsby, 1986) where animals are said to have mild, moderate and severe nematode infestation if their faecal egg counts are less than 500, 500-1000 and more than 1000, respectively.

Table 1. Physical characteristics of the animals.

Identity	Camel	Donkey	Total
Sex			
Male	36	1	37
Female	46	25	71
Age			
Infant	9	1	10
Juvenile	29	0	29
Sub-adult	7	1	8
Adult	37	24	61

Data management and analysis

The data collected from the study area was entered into Microsoft Excel spread sheet and the data was coded appropriately and analyzed using SPSS version 16 statistical software. Analysis Of variance (ANOVA) test was applied to test the statistical association existing among the risk factors such as species, sex, age and body condition scoring with the presence of the infection.

RESULTS

The total number of donkeys was 26, while camels were 82 giving a total of 108 animal samples analyzed. Number of positive cases for Strongyle EPG was 48 for camels and 11 for donkeys giving a prevalence of 58.5% for camels and 42.3% for donkeys. The mean EPG count was 1056 for camels and 323 for donkeys. The number of positive cases for *Anoplocephalidae* family of cestodes was 15 for camels and 4 for donkeys, giving a mean count of 74.4, prevalence of 18.3% in camels and 27, prevalence of 15.4% in donkeys (Table 2). Lung worm larvae reported *Dictyocaulus arnfieldi* in donkeys with 4 cases at a mean count of 0.4 and a prevalence of 15.4%. While *Dictyocaulus cameli* recorded 24 cases with a mean count of 1 at a prevalence of 29.3%.

Coccidia reported *Eimeria cameli* in camels with 9 cases in camel at a mean count of 34 at a prevalence of 11%, while *Eimeria leuckarti* in donkeys reported 1 case with a mean count of 11.5 at a prevalence of 3.85%.

Mixed infections were reported. Infections with one parasite species were 22 (26.8%) in camels and 8 (30.8%) in donkeys. Infections with 2 parasite species were 24 (29.3%) in camels and 6 (23%) in donkeys. Infections with 3 parasite species were 7 (8.5%) in camels and none in donkeys (Table 3).

Level of infection

Animals were said to have mild, moderate and severe

Table 2. Prevalence of endo parasites of donkeys and camels from Karamoja sub-region, Uganda.

Parasite/animal (mean EPG count)	No. positive camel/donkey	Camel (n = 82)	Donkey (n = 26)	Total
Strongyle eggs (EPG)	48/11	1056 (58.5%)	323 (42.3%)	1379
Dictyocaulus arnfieldi	0/4	N/A	0.4 (15.4%)	0.4
Dictyocaulus cameli	24/0	1 (29.3%)	N/A	1
Anoplocephalidae	15/4	74.4 (18.3%)	27 (15.4%)	101.4
Eimeria cameli	9/0	34 (11%)	N/A	34
Eimeria leuckarti	0/1	N/A	11.5 (3.85%)	11.5
Trematodes	5/0	12 (14.6%)	0	12

Table 3. Prevalence of various parasitic infections in camels and donkeys.

Infection status	Camels	Donkeys	Total
Infection with one species	22 (26.8%)	8 (30.8%)	30 (27.8%)
Infection with two species	24 (29.3%)	6 (23%)	30 (27.8)
Infection with three species	7 (8.5%)	0	7 (8.5%)
Infection with four species	0	0	0
General infection rate	51 (62.3%)	14 (53.8%)	65 (60.3%)

Table 4. Level of infection of strongyles in different age groups of donkeys and camels.

Age groups	Mild	Moderate	Severe	Total
Donkeys				
Infant	0	0	1 (3.85%)	1 (3.85%)
Juvenile	0	0	0	
Sub-adult	0	0	1 (3.85%)	1 (3.85%)
Adult	6 (23%)	2 (7.7%)	1 (3.85%)	9 (34.6%)
Camels				
Infant	0	0	4 (4.9%)	4 (4.9%)
Juvenile	5 (6%)	3 (3.7%)	4 (4.9%)	12 (14.6%)
Sub-adult	1 (1.2%)	1 (1.2%)	3 (3.7%)	5 (6%)
Adult	7 (8.5%)	4 (4.9%)	16 (19.5%)	27 (33%)

nematode infestation if their faecal egg counts are less than 500, 500-1000 and more than 1000, respectively (Table 4). Statistical analysis revealed a significant difference between the mean Strongyle EPG between the two districts, Moroto and Amudat (p=0.000), *Eimeria* EPG (p=0.018) and Trematode EPG (p=0.048), for the two districts; Mean Strongyle EPG Moroto (1281.8), Amudat (260); Mean *Eimeria* EPG Moroto (10.6), Amudat (60.0); Mean Trematode EPG Moroto (0.30), Amudat (0.25). The mean Strongyle EPG between species, camel and donkey was significant at p=0.020. Mean *Eimeria* EPG was statistically significant for location (p=0.018) and sex (p=0.017). Mean Trematode EPG was significant for location (p=0.048) and sex (p=0.006).

DISCUSSION

Lungworm infection characterized *D. cameli* of camels and *D. arnfieldi* as the parasite of horses and donkeys. *D. arnfieldi* has donkey as its normal host. While pathogenicity is limited in the donkey, the parasite may provoke severe clinical lungworm disease in the horse.

The family *Anoplocephalidae* comprises seven species of cestodes that inhabit the gastro-intestinal tract of camels and donkeys as adults or eggs found in the faeces. These include: *Moniezia expanza, Moniezia benedeni*, *Stilesia globipunctata*, *Stilesia vittata*, *Avitellina centripunctata, Avitellina woodland* and *Thysaniezia ovilla.*

Ugandan camels (*Camelus dromedaries*) were infected with *E. cameli* (11%) while 3.85% donkeys had *Eimeria leuckarti*. It is believed that the infection of coccidia is concern mainly with young horses and donkeys, especially foals. Although, it has been reported that *E. leuckarti* is more prevalent among young foals and only occasionally is it detected in adult animals (Souza et al., 2009). Watering devices should be protected from faecal contamination. Diagnosis is based on clinical signs and the demonstration of high numbers of oocysts in the diarrhoeic faeces. Cystic structures containing immature oocysts occur in the intestinal mucosa. In the present study, in all the cases, no clinical signs were documented in the animals excreting *E. leuckarti* oocysts, while there were some doubt about the pathogenicity of *E. leuckarti*, diarrhea of several days duration, and acute massive intestinal hemorrhage leading to rapid death have been described in foals (Radostits et al., 2006). However, the infection is rarely evidenced by clinical signs. Difficulties in the diagnosis of coccidia infection in camels, horses and donkeys cause the parasitosis not to be diagnosed in a routine coproscopical examination.

Trematodes were detected in 5 camels as *Fasciola gigantica*. Trematodes whose eggs may appear in the faeces including: *Fasciola hepatica* (liver flukes), *Fasciola gigantica* (giant liver fluke), *Dicrocoelium* spp. (liver flukes), *Eurytrema pancreaticum* (pancreas fluke), *Schistosoma bovis* and *Schistosoma mattheei* (blood flukes).

Strongyle eggs were quantified to determine egg per gram of faeces (EPG). Strongyles were taken as a group, speciation of the nematodes by ovaculture was not done. All the camels and donkeys were in good health and body condition except one sick donkey in Moroto which was in poor body condition. For this reason, autopsy was not done and hence adult worms were not studied. Therapy and prophylaxis for all the above mentioned helminth parasites is the same as for cattle.

Veterinary health care provision in Uganda does not cater for donkeys and camels at all. No veterinary intervention for these animals in Uganda is available at all. Yet these animals are kept in arid and mountainous areas of Karamoja and Sebei sub-region in North-eastern Uganda. They are kept for milk, meat, dowry, prestige and carriage.

Mixed infections were encountered comprising of double infections or triple infections of Strongyle, coccidian, cestode, trematode and lungworms. The challenge of mixed infections exacerbates the compromised health condition of the animal which could result in debilitation and death of the animal.

The number of eggs per gram can be calculated by counting the number of eggs within the grid of each of the 2 chambers, ignoring those outside the squares, then multipling the total by 50; this gives the eggs per gram of faeces (e.p.g.). Reading the count should not be delayed beyond the recommended time as the flotation fluid may distort or destroy delicate eggs. Therefore, it is advisable to only process a few samples at a time.

When interpreting McMaster results, it must be remembered that a number of factors can influence the occurrence, recognition or numbers of helminth eggs found in a faecal sample. In particular, the number of eggs is not necessarily indicative of the number of worms present. Reasons for this include: Eggs are produced only by fertile adult female (or hermaphrodite) worms and will, therefore, be absent in immature or single sex infections. The daily output of eggs by fertile females is influenced by host-physiological factors such as stress or lactation (increased) or immunity (decreased). Chemotherapy can also affect egg-production e.g. corticosteroids (increased) or sub-lethal anthelmintic doses (decreased). Some food-stuffs may have a similar effect e.g. tannin-rich forages (decreased). The concentration of eggs (per gram of faeces) is influenced by the daily volume of faeces being produced by the host, the rate of passage by the ingesta through the intestine, and the distribution of eggs throughout the faecal mass. Some types of eggs are heavier than others and may not float well in solutions of lower specific gravity (e.g. *Fasciola*). Some eggs from different species are indistinguishable (particularly trichostrongylids and strongylids). This complicates clinical interpretation as some species (e.g. *Haemonchus*) produce many more eggs per day than others (e.g. *Ostertagia*).

The mean EPG was statistically significant for male and female animals for *Eimeria* (p=0.017) and Trematodes (p=0.006). Although in this study, mean EPG *Eimeria* was higher in males (68.6) than females (12.1), and Trematodes male (0.34) and females (0). Female animals are ideally more prone to helminth infections physiologically because of stress, pregnancy and lactation conditions which compromise their immunity. The mean Strongyle EPG was 1056.1 in camels and 323.1 in donkeys. The mean Strongyle EPG between species, camel and donkey was significant at (p=0.020). It could be explained that donkey is a hardier animal, closer to wildlife species. Hence, it has a stronger resistance to infections naturally, more tendency as a reservoir host. However, fewer donkeys than camels were sampled in this study.

Conclusion

Helminthes infection is endemic in camels and donkeys in Uganda. A wide range of species of helminthes that plague donkeys and camels was detected in Karamoja sub-region, Uganda. The observation of multiple infections with high prevalence and high overall EPG suggest the presence of favorable environmental conditions for survival, infection and perpetuation of helminthes of camels and donkeys in Uganda. There is complete lack of veterinary service provision to these

animals and lack of awareness of animal welfare. Information on the different aspects of parasitology of these animals is also limited. Production and productivity of camels and donkeys in Uganda is compromised by the little or no veterinary care and welfare of these animals.

Conflict of interest

The authors have not declared any conflict of interest

ACKNOWLEDGEMENT

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REFERENCES

Anvari-Tafti M, Sazmand A, Hekmatimoghaddam S, Moobedi I (2013). Gastrointestinal helminths of camels (*Camelus dromedarius*) in center of Iran. Trop. Biomed. 30(1):56-61.

Arslan MO, Umur S (1998). The helminth and Eimeria (Protozoa) species in horse and donkey in Kars. Acta. Parasitol. Turcica. 22:180-184.

Atawalna J, Emikpe BO, Sallah EK, Shaibu W, Folitse RD (2015). The Health Problems, Gastrointestinal and Blood Parasites Commonly Associated With Donkeys in the Upper East Region of Ghana. Afr. J. Biomed. Res. 18:37-41.

Ayele CF (2006). Principal health problems of donkeys in Dugda Bora district of Ethiopia. In: Proceedings of the 5th International Colloquium on Working Equines Thethe Future for Working Equines. Eds: Pearson AA, Muir CC, Farrow MM. The Donkey Sanctuary, Sidmouth. pp. 162-168.

Bakirci S, Çirak VY, Gülegen E, Karabacak A (2004). Parasites found by faecal examinations in horses in the Gemlik Military Stud Farm. Acta. Parasitol. Turcica. 28:35-37.

Demir S, Tinar R, Kaplan A (1995). Helminths obtained from a donkey. Acta. Parasitol. Turcica. 19:119-123.

Fikru R, Reta D, Teshale S, Bizunesh M (2005). Prevalence of equine gastrointestinal parasites in western highlands of Oromia, Ethiopia. Bull. Anim. Health Prod. Afr. 53:161-166.

Forety WJ (2001). Veterinary Parasitology. Reference Manual, Fifth Edition, Iowa State University Press.

Getachew AM, Trawford AF, Feseha G, Reid SWJ (2010). Gastrointestinal parasites of working donkeys in Ethiopia, Trop. Anim. Health Prod. 42:27-33.

Gordon HM, Whitlock HV (1939). A new technique for counting nematode eggs in sheep faeces. J. Counc. Sci. Ind. Res. 12:50-52.

Ismail AA, Seri HI, Ahmed NK, El Tigani-Asil El TA, Bashar AE, Abakar AD (2016). A Survey of Seasonal Gastrointestinal Parasitic Infections in Donkeys from a Semiarid Sub-Saharan Region, Sudan. Hindawi. J. Pathog. P 8.

Ozer E, Küçükerden N (1992). Helminth and *Eimeria sp*. found in equids in Elazig and region. Doga Tr. J. Vet. Anim. Sci. 17:217-221.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2006). A text-book of the diseases of cattle, horses, sheep, pigs and goats. Veterinary Medicine E-Book, 10th Edition. Vet. med. Saunders.

Sonja M, Rosina CK, Susan AM (2000). Prevalence and Biodiversity of helminth parasites in donkey from South Africa. J. Parasitol. 86:756-762.

Soulsby E (1982). “Helminths, Arthropods and Protozoa of Domestic Animals,” Bailliere TinDall, Toronto.

Soulsby EJL (1986). Helminthes, Arthropods and Protozoa of Domesticated Animals (7thedn) Bailliere Tindall London. 167-174

Souza PNBD, Bomfimb TCB, Huber F, Abboud LCS, Gomes RS (2009). Natural infection by Cryptosporidiumsp. Giardia sp. and Eimeria leuckarti in three groups of equines with different handlings in Rio de Janeiro. Brazil. Vet. Parasitol. 160:327-333.

Sumbria D, Moudgil AD, Singla LD (2014). Equine Piroplasmosis: Current status. Veterinaria 1(1):9-14.

Sumbria D, Singla LD (2015). Recent Diagnostic and control approaches in Equine piroplasmosis. Veterinaria 2(1):1-6

Tsegaye B, Chala A (2015). Prevalence of endoparasitic helminths of donkeys in and around Haramaya district, Eastern Ethiopia. JVMAH 7:221-224.

Yoseph S, Smith DG, Mengistu A, Teklu F, Firew T, Betere Y (2005). Seasonal variation in the parasite burden and body condition of working donkeys in East Shewa and West Shewa regions of Ethiopia. Trop. Anim. Health Prod. 37:35-45.

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Full Length Research Paper

Eukaryotic expression and characterization of BHV-1 glycoprotein D (gD) as a potential diagnostic antigen

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Bovine herpesvirus-1 (BHV-1) causes Infectious bovine rhinotracheitis/Pustular vulvovaginitis in cattle. Glycoprotein D (gD) of BHV-1 represents a major component of the viral envelope and is a dominant immunogen. gD encoding gene was expressed in baculovirus-insect cell system. Viral genomic DNA extracted from BHV-1 grown on Madin-Darby Bovine Kidney (MDBK) cell monolayer was used as a template for PCR amplification of gD gene (1255 bp). Gel purified gD gene was used for directional cloning into pENTR/SD/D Directional TOPO vector to produce entry clone. Recombinant plasmids were screened by PCR and restriction enzyme (RE) digestion for gD gene insert. Endotoxin free purified plasmids were then subjected to LR recombination reaction with baculovirus linear DNA. LR recombination mix was transfected into Sf-9 cells and observed for appearance of cytopathic effects (CPE). Recombinant virus was serially passaged for 3 more generations and the 4th passage viral stock was used to infect fresh Sf-9 cells for gene expression study. Recombinant gD protein was immunoprecipitated and when subjected to SDS-PAGE and western blot analysis protein band of ~70 kDa was detected consistently. The recombinant gD protein was further weakly confirmed by dot-ELISA indicating its limited potential as a coating antigen in gD-based diagnostic ELISA.

Key words: Bovine herpesvirus-1 (BHV-1), Madin-Darby Bovine Kidney (MDBK) cells, Sf-9 cells, pENTR TOPO vector, Baculovirus mediated gene expression, glycoprotein D, eukaryotic expression,

INTRODUCTION

Bovine herpesvirus type-1 (BHV-1) causes various diseases worldwide (Biswas et al., 2013; Levings and Roth, 2013). It is the most common viral agent found in the semen of bovines (Saminathan et al., 2016) and can be transmitted through natural or artificial insemination (Godhardt-Copper et al., 2009). Infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) (Davis et al., 2014; Majumder et al., 2014) are few of the most important diseases which cause huge economic loss to dairy industry due to abortion and drop in milk production (Saminathan et al., 2016). BHV-1, large double stranded DNA virus (Guo et al., 2015; Kirchhoff et al., 2014), is one of three viruses responsible for the bovine respiratory disease complex (BRDC) associated with reduced immunity (Kirchhoff et al., 2014). It causes a latent infection in sensory neurons but can reactivate following an increase in corticosteroid level (da Silva et al., 2013). Apart from IBR and IPV, BHV-1 is

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also responsible for many other clinical manifestations namely abortion, endometritis, infertility, mastitis, rhinotracheitis, encephalitis (Davis et al., 2014) conjunctivitis (Levings, 2012), balanoposthitis (Kaur et al., 2013; Muylkens et al., 2007; Saminathan et al., 2016) and is fatal to newborn calves (Engels and Ackermann, 1996). 12 envelope glycoproteins have been identified and seven among them, namely gB, gC, gD, gE, gH, gK and gL are known to be involved in viral attachment (Drummer et al., 2014; Biswas et al., 2013). gD gene is contiguous with gI gene with 141 bp region separating their two open reading frames (Chowdhury and Sharma, 2012). gB, gC, and gD are reported to be the major envelope glycoproteins recognized by both cellular and humoral immunity (Blanc et al., 2012) and consequently major BHV-1 immunogens (Collins et al., 1985).

All three glycoproteins are used in vaccine production and induce higher titer neutralizing antibodies with gD producing the highest titer (Dummer et al., 2014; Blanc et al., 2012). gD is reported to be potential candidate for production of subunit vaccines (Majumder et al., 2014) and its expression is limited to the alpha herpesvirus, the largest subfamily of herpesvirus (Connolly et al., 2001**;** Grauwet et al., 2014). Glycoproteins gB and gD participate in viral replication (Tikoo et al., 1995). gD abundance in the viral envelope and its role in initial stage of replication define it as a good target of the host immune response (Drummer et al., 2014) making it immunodominant and a good candidate for vaccine (Blanc et al., 2012; Majumder et al., 2014). These biological properties make the three glycoproteins powerful antigens in the study of BHV-1 immune response (Abdelmagid et al., 1998).

BHV-1 infection in India is endemic in nature and ELISA based diagnostic tests are being used commonly for seroprevalence studies in large population against several diseases including BHV-1. Most of these assays require viral antigen in bulk and recently diagnostic ELISAs based on recombinant protein are being developed as large scale production of recombinant viral protein is possible in safe and cost effective manner. With the recent development of marker viral vaccines, individual viral protein antigens will become a powerful tool for diagnostic evaluation of immune response as well as to differentiate infected animals from vaccinated ones (Kit and Kit, 1991; Drummer et al., 2014; Muylkens et al., 2007). Various expression system including insect cells have been developed for expression of different proteins of pathogens (Saminathan et al., 2016).

Keeping these points in view, this study was aimed at cloning for eukaryotic expression of BHV-1 gD and characterization of the expressed recombinant protein.

MATERIALS AND METHODS

Cell, virus and antibodies

MDBK cells were propagated in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and containing sodium bicarbonate, 2 mM L-glutamine, 10 mM HEPES. Sf-9 (*Spodoptera frugiperda*) cells were propagated in Grace's Insect Medium supplemented with 10% FBS (Invitrogen). Sf-900^{TM} II Serum Free Media (Invitrogen) also supplemented with 10% FBS was used in later stage for protein expression study. A solution having both antibiotic and antimycotic activity (100×, HIMEDIA) containing amphotericin B, penicillin and streptomycin was added to the medium.

25 cm^2 cell culture flasks each containing (85-90%) monolayer of MDBK cells were used to propagate BHV-1 isolate IBR 216 II (available in the laboratory) at multiplicity of infection (m.o.i.) 0.1 and the virus was serially passaged 4 to 5 times.

Anti-sera against BHV-1 and Anti-gD mouse monoclonal antibody were used in this study; the first was available in animal biotechnology department and the second was procured from Biodot laboratory, New Delhi.

DNA extraction of BHV-1 and PCR

AuPrep GEN DNA Extraction kit (Life Technologies) was used to extract genomic DNA from Bovine herpesvirus-1 passaged in MDBK cell line. gD gene was amplified from 5 µl of template DNA by PCR. CACC overhang immediately preceding initiation codon sequence (ATG) and kozak sequence were two important features of the forward primer while the reverse primer was designed simply to amplify the gD gene from the codon immediately preceding the stop codon. The gD Forward primer was: 5' CACCATGGAAGGGCCGACATTG 3' and the reverse primer was 5' CCCGGGCAGCGCGCTGTAGTTG 3'. The actual size of gD gene is 1251 bp but addition of 4 bp, CACC, overhang increased the size of amplified product to 1255 bp.

PCR was carried in 50 µl volume made of 150 ng of DNA, 1 µl of each of primers at 25 pmol concentration, 6 µl of deoxynucleoside triphosphates (dNTPs) mix at 2.5 mM, 1.5 µl of magnesium sulphate at 50 mM concentration, 3 µl of dimethyl sulfoxide, 5 µl of enhancer solution, Pfx platinum buffer 1X and Pfx platinum DNA polymerase at 1.5 U. The conditions of PCR were: 94°C for 2 min, then 35 cycles of 94°C for 30 s, 50°C for 40 s, 72°C for 2 min and lastly the final extension at 72°C for 10 min in thermal cycler (Eppendorf). PCR product was visualized using ethidium bromide post stained agarose gel (1.5%) and its molecular weight was determined by comparing it to the 100 bp ladder (Fermentas).

Cloning of BHV-1 gD gene

A QIA Quick gel extraction kit (Qiagen) was used to purify the PCR product followed by directional cloning of the PCR product in pENTR^{TM}/SD/D-TOPO® vector (Invitrogen) as per the manufacturer's guideline. 2 µl of the cloning reaction were used to transform chemically competent *E. coli* host strain TOP10 cells and LB agar plates with 100 µg/ml of kanamycin were used for selection of transformed colonies. Selected clones (5 to 6 numbers) were grown overnight in LB broth at 37°C in a shaker incubator in presence of kanamycin (100 µg/ml). Recombinant plasmids were extracted from the broth culture by alkaline lysis method (Sambrook et al, 1990). The presence of BHV-1 gD gene and its correct orientation in the expression vector was confirmed by Touchdown/Colony PCR followed by PCR from extracted plasmids and the recombinant plasmid digestion with *Asc* I and *Not* I restriction enzymes. Gen Elute Endotoxin-free Plasmid Midiprep kit (SIGMA-ALDRICH) was used to prepare the plasmid DNA (free from endotoxin) which was then used as entry clone for recombination with baculovirus genomic DNA before transfection of the insect cells.

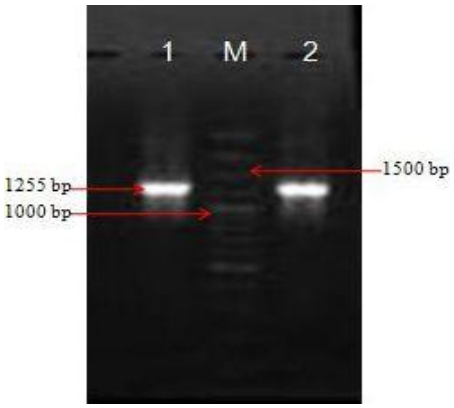

Figure 1. PCR amplified BHV-1 complete gD gene from genomic DNA. Lane 1, 2, Amplified gD gene positive samples; Lane M, 100 bp plus DNA ladder.

Transfection in Sf-9 cell line

The transfer of the gene of interest (gD) into the BaculoDirect™ Linear DNA (Invitrogen) was mediated by LR recombination reaction. A transfection mixture was prepared using Cellfectin reagent and LR recombination mixture. Sf-9 cells in log phase with the viability greater than 95% were used for each transfection. Approximately 8×10^5 Sf-9 cells in 2 ml of complete growth medium were seeded per well in a six well plate followed by incubation at 27°C for one hour. Subsequently, in each well of Sf-9 cells, LR recombination mixture was added and again incubated. Selection of recombinant baculovirus encoding gD gene was done by adding 100 µM ganciclovir to the transfected Sf-9 cells. After 96 h of incubation at 27°C, cells were harvested upon observation of cytopathic effect. The P1 viral stock was used for 2 more passages up to P3. Sf-9 cells in 75 cm^2 cell culture flasks were infected with this P3 viral stock for gene expression study.

Ni-NTA column chromatography and immunoprecipitation

Ni-NTA Column chromatography which uses Ni-NTA superflow columns (Qiagen) and immunoprecipitation which uses Protein G Immunoprecipitaion kit (SIGMA) as recommended by the manufacturer (cell lysate (60 µl), Monoclonal antibody (5 µl) and 1X immunoprecipitation buffer "IP buffer" (535µl) were added to one spin column and cell lysate (60µl), Polyclonal antibody (15µl) and 1X IP buffer (525 µl) were added to another spin column to make the final volume 600 µl in each column. Both spin columns were incubated for 1 h at 4°C after which 30 µl of Protein-G-Agarose beads washed twice with 1X IP buffer and finally mixed with 50 µl of fresh 1X IP buffer were transferred into each spin column. Spin columns were then centrifuged at 12,000 rpm for 20 s at 4°C and the effluent were discarded. Beads were again washed twice with 1X IP buffer and 0.5 M NaCl by centrifugation at 12,000 rpm for 20 s followed by 4 times washing with 1X IP buffer. The last washing was performer with 0.1X IP buffer) were used to purify the recombinant gD protein from the P3 virus infected Sf-9 cells. Purified protein samples thus obtained were subjected to SDS-PAGE and western blot analysis.

SDS-PAGE and Western blot analysis

Sf-9 cells infected by recombinant baculovirus and non-infected ones, were harvested and prepared for SDS-PAGE by the method of sonication where centrifugation at 5000 rpm produced the pellet which was resuspended in 500 µl PBS and 5 µl protease inhibitor followed by sonication thrice at 5 microns for 10 s with a gap of 20 s each. Samples were then centrifuged again at 5000 rpm for 10 min and the supernatants were collected into new tubes for SDS-PAGE analysis. Samples along with the protein marker were run on 10% SDS-PAGE gel followed by staining with Coomassie Brilliant Blue (Sambrook et al., 1989). An unstained gel was blotted onto a PVDF membrane for Western blot and a SNAPid system (Millipore) was used for development. A solution of PBS with 0.1% Tween-20 and 1% BSA was used to block the membrane for 10 min at room temperature. The membrane was respectively incubated at room temperature with monoclonal antibody against BHV-1 gD and Goat anti-mouse HRP conjugate, the first at 1:300 dilution for 10 min followed by washing thrice with (PBS + 0.1% Tween 20) buffer but the second at 1:100 dilution for 10 min also followed by washing thrice with PBS. To develop the blot, DAB solution made of 1mg/ml diamino benzidine and 10 µl/ml hydrogen peroxide in PBS was used.

Dot ELISA

The following were used for Dot-ELISA: partially concentrated BHV-1, MDBK cell lysate, immunoprecipitated gD, Sf-9 infected cell lysate, non-infected Sf-9 cell lysate, PBS and blank. On dot-ELISA strip, 2 µl of each sample was spotted and it was kept for drying at 37°C for 1 h. 1% BSA was used for blocking for 30 min followed by 5 times washing with a solution of PBS containing 0.01% Tween-20, incubation with polyclonal antibody as primary antibody at 1:200 dilution and again washing once. The last incubation for 1 h at 37°C was done with secondary antibody, goat anti-rabbit HRP conjugate, diluted at 1:500 followed by washing once. DAB and hydrogen peroxide were used to develop the blot as described previously.

RESULTS

Cloning of gD gene and screening of the recombinant plasmids for presence of the insert

Genomic DNA of BHV-1 was isolated from partially purified virus and the complete gD gene (1255 bp) was amplified by PCR using specific primers to obtain gD gene fragments containing CACC base sequence at 5'end just preceding the initiation codon (Figure 1). Blunt end PCR amplified gene products were gel purified and directly used for cloning into PENTR/SD/D-directional TOPO cloning vector. Screening clones for gD gene insert was done by touch down PCR for presence of gD gene insert where PCR product of 1255 bp could successfully be amplified from recombinant plasmids. Clones having gD gene insert were grown for plasmids isolation and again the presence of gD gene insert from the purified recombinant plasmids was confirmed by amplification of 1255 bp product (Figure 2). The confirmation of complete gD gene's presence was also possible by digesting plasmids with *Asc* I and *Nco* I restriction enzymes which released 1297 bp fragment having additional 42 bp from multiple cloning site region of the plasmid (Figure 3).

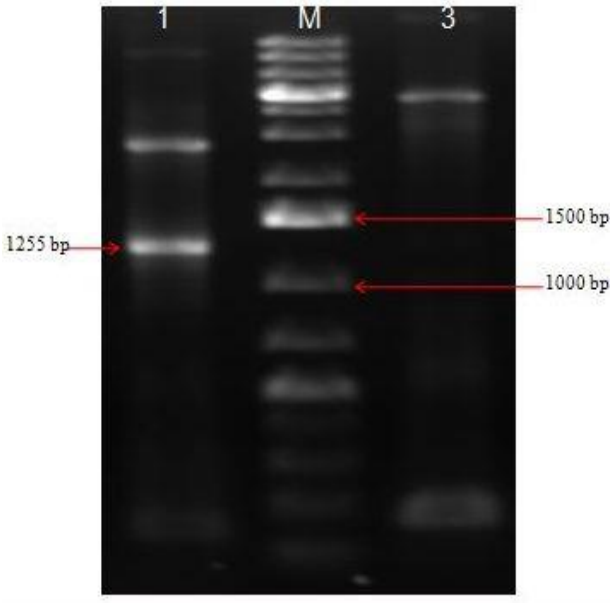

Figure 2. PCR amplification of gD gene (1255 bp) with gD gene specific F & R primers from pENTR-TOPO gD clones. Lane M, 1Kb DNA ladder;Lane 1, 3, pENTR-TOPO clones; Lane 1, positive clone.

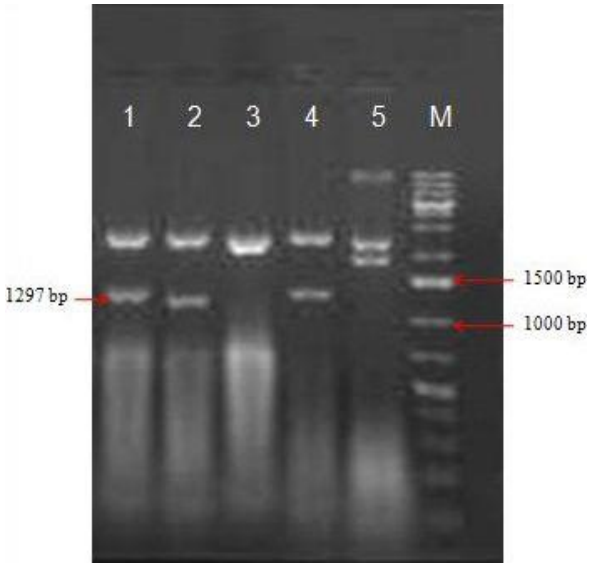

Figure 3. Release of gD gene insert by *Asc* I and *Nco* I RE digestion of the recombinant plasmids. Lane M, 1 kb DNA Ladder plus; Lane 1-5, Recombinant plasmids; Lane 1, 2, 4: Plasmids carrying gD gene insert.

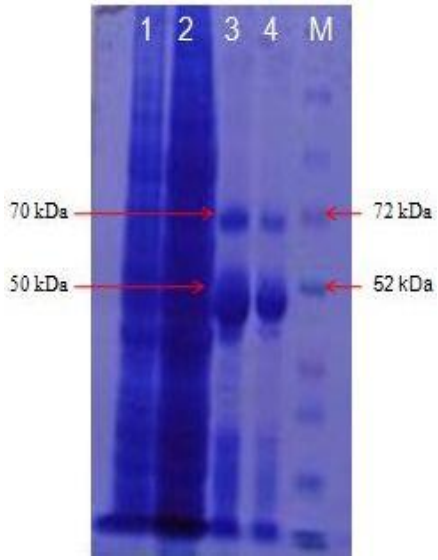

Figure 4. SDS-PAGE analysis of immunoprecipitated recombinant gD protein and partially purified BHV-1. Lane 1, Non infected Sf-9 cell lysate; Lane 2, Sf-9 infected cell lysate; Lane 3, Immunoprecipitated BHV-1; Lane 4, Immunoprecipitated gD; Lane M, Protein ladder.

Analysis of recombinant protein

The optimum duration for maximum protein expression was 72 h post infection as revealed by Western blot analysis. Immunoprecipitated expressed protein (from 72 h post infected cell lysate) was analyzed by SDS-PAGE where two bands of ~70 kDa and ~50kDa were observed (Figure 4). On western blot analysis with polyclonal anti BHV-1 serum, strong reaction of protein was observed with band of ~70 and 50 kDa (Figure 5). On SDS-PAGE together with western blot analysis (Figure 6a), gD protein was detected from 24 to 144 h post infection from Sf-9 cells, however the maximum expression being observed at 72 h (Figure 6b).

When expressed protein purified by immunoprecipitation was run on SDS-PAGE, a band of ~70 kDa and an additional band of ~50 kDa were observed. On western blot analysis using polyclonal anti BHV-1 serum, strong reaction of protein was observed with these bands (Figure 5 and 7), however the reaction being intense with polyclonal anti-BHV-1 compared to monoclonal.

The recombinant gD protein which appeared to be of a molecular mass of ~70 kDa was absent from uninfected cells. On western blot analysis, authentic gD (~70 kDa) and a polypeptide with an apparent molecular mass of ~70kDa were recognized by polyclonal antibody respectively in MDBK cells infected with BHV-1 and Sf-9 cells infected with recombinant baculovirus. This suggests that the recombinant gD is equivalent to the authentic gD.

Dot ELISA

The reaction of both immunoprecipitated gD protein and

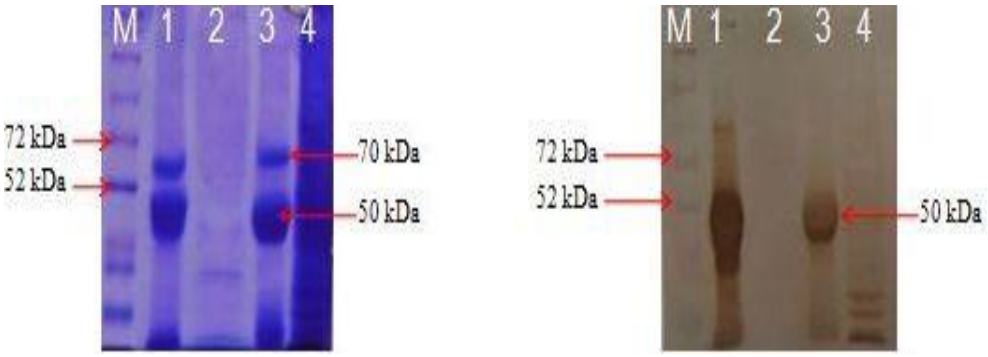

Figure 5. SDS-PAGE (left) and Western blot with Polyclonal antibody (right) analysis of the recombinant gD after 72 h of infection. Lane M, Protein ladder; Lane 1, Immunoprecipitated gD; Lane 2, Semi purified BHV-1; Lane 3, Immunoprecipitated gD; Lane 4, Non infected Sf-9 cell lysate.

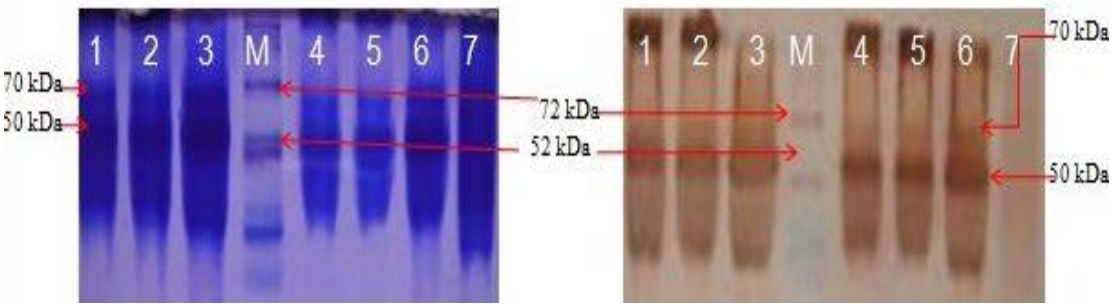

Figure 6. SDS-PAGE (left) (a) and Western blot with polyclonal antibody (right) (b) analysis of immunoprecipitated recombinant gD at different intervals of time after infection. Lane 1, Immunoprecipitated gD 24 h post infection; Lane 2, Immunoprecipitated gD 48 h post infection; Lane 3, Immunoprecipitated gD 72 h post infection; Lane M, Protein ladder; Lane 4, Immunoprecipitated gD 96 h post infection; Lane 5, Immunoprecipitated gD 120 h post infection; Lane 6, Immunoprecipitated gD 144 h post infection; Lane 7, Noninfected Sf-9 cell lysate.

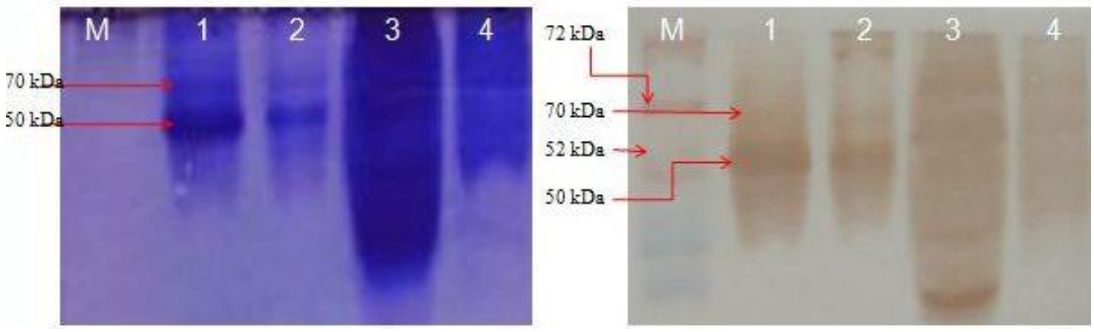

Figure 7. SDS-PAGE (left) (a) and Western blot (right) (b) analysis of the recombinant gD with monoclonal antibody. Lane 1: protein ladder; Lane 2, Immunoprecipitated gD; Lane 3, Immunoprecipitated BHV-1; Lane 4, Sf-9 infected cell lysate; Lane 5, Non infected Sf-9 cell.

gD protein recovered from infected Sf-9 cell lysate as shown in Figure 8, was also detected in Dot ELISA. As revealed by dark brown spots, the native protein gD recovered from both partially purified BHV-1 and BHV-1 infected cell lysate appeared to have fair reactivity with polyclonal antibody.

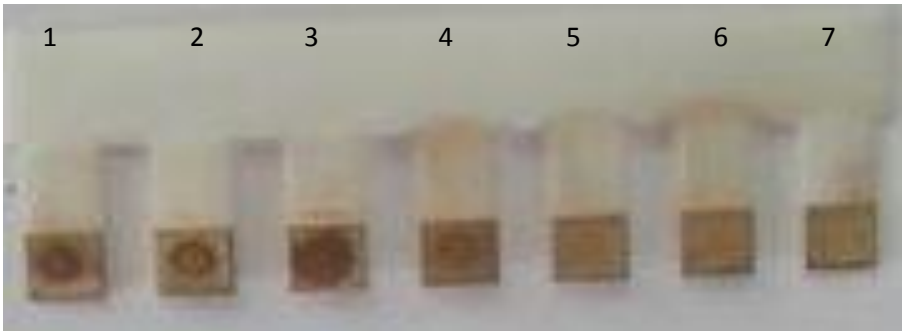

Figure 8. Dot ELISA (with polyclonal antibody) for detection of reactivity of the expressed gD recombinant protein. 1, Partially purified BHV-1; 2, MDBK infected cell lysate; 3,Immunoprecipitated gD; 4, Sf-9 infected cell lysate; 5, Non infected Sf-9 cell lysate; 6, PBS; 7, Blank.

DISCUSSION

As Abdelmagid et al. (1998) reported earlier, 72 h post infection was the optimum period for protein expression. Two bands of 70 and 50 kDa for the recombinant gD proteins were observed as revealed by SDS-PAGE and western blot analysis. Parker et al. (1991) reported to have expressed BHV-1 gp IV (D) of an apparent molecular mass of 63 kDa by recombinant baculovirus and its lower apparent molecular mass compared to the native protein indicated the incomplete glycosylation. The expressed protein appeared to be antigenically intact in this study as its reaction with polyclonal and monoclonal antibodies specific to gD was comparable to that of gD from BHV-1. These findings are in accordance with the work of Parker et al. (1991) and supported by the fact that Sf-9 cell are higher eukaryote and therefore having proper posttranslational modification (Hu, 2005).

The reactivity of anti gD monoclonal antibody with recombinant gD protein was found to be lower as compared to polyclonal antibody suggesting that the expressed protein is not being recognized properly by monoclonal antibody used as revealed by both dot ELISA and Western blot. This may result from modification or loss of epitope conformation due to protein denaturation during sample preparation together with or improper glycosylation as baculovirus infected cell display inability to correctly process some proteins (Hu, 2005) due to the fundamental nature of insect glycoprotein processing pathways (Jarvis, 2003). The poor reactivity of monoclonal antibody with a recombinant protein due to loss of native conformation of baculovirus expressed protein was also reported by Abdelmagid et al (1998) in the case of glycoprotein B (gB).

With both monoclonal and polyclonal antibodies, protein bands of 70 and 50 KDa were consistently detected in SDS-PAGE and western blot analysis. Blanc et al (2012) reported to have expressed glycoprotein D (gD) of 71 kDa and according to Van Donkersgoed et al. (1994) glycoprotein D (gD) of 71 kDa containing both N-linked and O-linked Oligosaccharides is synthesized first as a partially glycosylated precursor of 63 KDa.

Conclusion

BHV-1 gD could be successfully expressed using baculovirus based vector and insect cell system which was visualized as protein bands of 70 and 50 KDA in SDS-PAGE and Western blot assays. The expressed gD is equivalent to the authentic gD and has fair potential to be used as a diagnostic antigen for detection of BHV-1 specific antibodies.

Conflict of interest

The authors have not declared any conflict of interest.

REFERENCES

Abdelmagid OY, Mansour MM, Minocha HC, van Drunen Littel-van den Hurk S (1998). Evaluation of baculovirus-expressed bovine herpesvirus-1 (BHV-1) glycoproteins for detection and analysis of BHV-1-specific antibody responses. Vet. Microbiol. 61:249-259.

Biswas S, Bandyopadhyay S, Dimri U, Patra HP (2013). Bovine herpesvirus-1 (BHV-1)–a re-emerging concern in livestock: a revisit to its biology, epidemiology, diagnosis, and prophylaxis. Vet. Q. 33(2):68-81.

Blanc AM, Berois MB, Tomé LM, Alberto L, Epstein AL, Arbiza JR (2012). Induction of humoral responses to BHV-1 glycoprotein D expressed by HSV-1 amplicon vectors. J. Vet. Sci. 13(1):59-65.

Connolly SA, Whitbeck JC, Rux AH, Krummenacher C, Cohen GH, Eisenberg RJ (2001). Glycoprotein D homologs in herpes simplex virus type 1, pseudorabies virus, and bovine herpes virus type 1 bind directly to human HveC (nectin-1) with different affinities. Virol. 280(1):7-18.

Chowdhury S, Sharma B (2012). Transcription of gD and gI genes in BHV1-infected cells. J. Biosci. 37**:**971-977.

Collins JK, Butcher AC, Riegel CA (1985). Immune response to Bovine herpesvirus type 1 infections: Virus-specific antibodies in sera from infected animals. J. Clin. Microbiol .21:456-552.

da Silva LF, Kook I, Doster A, Jones C (2013). Bovine herpesvirus 1 regulatory proteins bICP0 and VP16 are readily detected in trigeminal

ganglionic neurons expressing the glucocorticoid receptor during the early stages of reactivation from latency. J. Virol. 11214-11222.
Davis KJ, Syam RS, Chauhan RS (2014). Epidemiological studies on infectious bovine rhinotracheitis (IBR) in different parts of India. Int. J. Live. Res. 4(5).
Dummer LA, Leite FBL, van Drunen Littel-van den Hurk S (2014). Bovine herpesvirus glycoprotein D: a review of its structural characteristics and applications in vaccinology. Vet. Res. pp. 45:111.
Engels M, Ackermann M, (1996). Pathogenesis of ruminant herpesvirus infections. Vet. Microbiol. 53:3-15.
Godhardt-Cooper JA, Zoromski J, Toohey-Kurth K (2009). Evaluation of a blocking enzyme-linked immunosorbent assay for serological diagnosis of Bovine herpesvirus1. J. Vet. Diagn Invest.. 21:523-526.
Grauwet K, Cantoni C, Parodi M, Andrea de Maria, Devriendt B, Pende D Moretta L, Vitale M, Favoreel HW (2014). Modulation of CD112 by the Alphaherpesvirus gD protein suppresses DNAM-1–dependent NK cell-mediated lysis of infected cells. Proc. Natl. Sci. Acad.111(45):16118-16123.
Guo L, Yanling Yang Y, Liu L, Liao P, Wen Y, Wu H, Cheng S (2015). A proteomic study of the differential protein expression in MDBK cells after bovine herpesvirus type 1 infection (BHV-1) strain treatment. Int. J. Clin. Exp. Med. 8(3):4204-4211.
HU Y (2005). Baculovirus as a highly efficient expression vector in insect and mammalian cells. Acta Pharmacol. Sin. 26 (4):405-416.
Jarvis DL (2003). Developing baculovirus-insect cell expression systems for humanized recombinant glycoprotein production.Virology 310:1-7.
Kaur G, Dwivedi P N, Verma R, Sharma NS (2013). Prevalence of Bovine herpesvirus-1 in cattle and buffaloes in Punjab. Vet. World 6(6):343-345.
Kirchhoff J, Uhlenbruck S, Goris K, Keil GM, Herrler G (2014). Three viruses of the bovine respiratory disease complex apply different strategies to initiate infection. Vet. Res. pp.45:20.
Kit M, Kit S (1991). Sensitive glycoprotein gIII blocking ELISA to distinguish between pseudorabies (Aujeszky's disease)-infected and vaccinated pigs. Vet. Microbiol. 28:141-155.
Levings RL (2012). "Generation and characterization of virus-neutralizing bovine monoclonal antibodies to bovine herpesvirus 1 glycoproteins gB, gC, and gD" Graduate theses and dissertations. Paper 12862.
Levings RL, Roth JA (2013). Immunity to Bovine herpesvirus 1: II. Adaptive immunity and vaccinology. Anim. Health Res. Rev. 14(1):103-123.
Majumder S, Pandey AB, Ramakrishnan MA (2014). Cloning and sequence analysis of glycoprotein D gene of bovine herpesvirus 1. Adv. Anim. Vet. Sci. 2(2S):19-22.
Muylkens B, Thiry J, Kirten P. Schynts F,Thiry E (2007). Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis Vet. Res. 38:181-209.
Sambrook J, Fritsch E, Maniatis T (1989). Molecular cloning (Vol. 2, pp. 14-9). New York: Cold spring harbor laboratory press.
Sambrook J, Fritsch EF, Maniatis T (1990). Molecular cloning: a laboratory manual, Ed 2nd.
Saminathan M, Rana R, Ramakrishnan MA, Karthik K, Malik YS, Dhama K (2016). Prevalence, diagnosis, management and control of important diseases of ruminants with special reference to Indian scenario. J. Exp. Bio. Agri. Sci. 4(3S).
Tikoo S, Campos M, Babiuk L (1995). Bovine herpesvirus 1 (BHV-1): Biology, pathogenesis, and control. Adv. Virus Res. 45:191-232.
Parker MD, Fitzpatrick DR, Zamb TJ, van den Hurk JV, Campos M, Harland R, Babiuk LA (1991). Expression of Bovine herpesvirus 1 glycoprotein IV by recombinant Baculovirus and analysis of its immunogenic propeerties. J. Virol. 65:263-71.
Van Donkersgoed J, Kowalski J, Van den Hurk JV, Harland R, Babiuk LA, Zamb TJ (1994). A subunit gIV vaccine, produced by transfected mammalian cells in culture induces mucosal immunity against bovine herpesvirus-1 in cattle. Vaccine 12:1295-302

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